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*

STUDIES ON THE KINETICS OF THE PARIETAL SECRETION OF THE STOMACH

BY

K. J. ÖBRINK

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CHAPTER 1

The Purpose of the Present Investigation.

Investigations concerning the gastric secretion are numerous and many results have appeared since the fundamental works of BEAUMONT (1833).

The greatest interest has been directed towards the secretion of hydrochloric acid, but in spite of many attempts to solve the question of the formation of this acid in the stomach, the biochemical mechanism of this physiological process is quite unknown. Several theories have been suggested — cf. HARVEY & BENSLEY (1912), ZIMMERMANN (1925), HOLLANDER (1943), DAVENPORT (1939, 1940, 1941, 1942, 1943 a), DAVENPORT & FISCHER (1940), BULL & GRAY (1945), CONWAY & BRADY (1947), DAVIES, LONGMUIR & CRANE (1947) — but none of them has been supported by sufficiently convincing experiments.

If an explanation of the HCl-formation is ever to be arrived at, thorough *quantitative* analyses of the secreting procedure must be performed. Such analyses are relatively rare and often carried out with unsatisfactory methods. This treatise will, therefore, attempt to furnish further information about the quantitative aspects involved, i. e. *the kinetic interrelations between stimulation and its effects.*

Strong evidence has been given for the suggestion that the parietal cells are the elements of the gastric mucosa which are responsible for the HCl-formation. (Whether or not the acid is formed within the cells or on their surfaces is not known.) If the secretion of HCl is to be as nearly as possible uncomplicated, the parietal cells alone should be stimulated. The stimulus seems normally to arise from the pyloric mucosa — EDKINS (1906), KOMAROW (1938), UVNÄS (1942, 1943, 1945 a, b).

POPIELSKI (1920) discovered that histamine strongly stimulated the secretion of gastric acid. Since that time no better secretagogue acting exclusively on the parietal cells has appeared. In

fact most authors deny a non-parietal stimulation by the histamine — cf. BABKIN (1944).

In the present investigation histamine was therefore used as a stimulator of the gastric secretion. In order to depress the other cell-groups as much as possible the operative technique proposed by HEIDENHAIN (1878, 1879) was used on dogs, i. e. denervated gastric pouches were prepared. In this way the peptic secretion was thought to be partially depressed.

It would first be of interest to know the histamine concentrations which stimulate gastric secretion. This special problem will be discussed from a kinetic point of view.

The relation between the histamine concentration and its action on the parietal cells will be investigated and also the relationship between secretion rate and acidity, chloride concentration, excretion capacity of dyestuffs etc.

As a new substance has recently been found in the intestinal mucosa which inhibits the parietal secretion — *enterogastrone* — it was thought desirable to investigate its action on the secretion during histamine stimulation.

Attempts have also been made to investigate the quantitative relationship between the state of secretory activity and the electric potential difference across the gastric mucosa. These investigations, which have lent support to the theory that there is some relation between the secretion rate and the potential difference, will, however, be continued and published separately elsewhere.

A great number of papers having a connection with this work could not be mentioned, and the reader is therefore referred to the monographs of CARLSON (1923), ROSEMAN (1927) and BABKIN (1944). Concerning special histamine problems the monograph of FELDBERG & SCHILF (1930) may be consulted and on the question of the excretion of dyestuffs the books of MATSUO (1934—1935) are valuable.

CHAPTER 2.

Procedure.

The experiments in the present paper were carried out on animals, predominantly *dogs*. In some few cases *cats* were used.

It is thought most convenient to give a rather detailed description

of the procedure in the dog experiments. Changes in this procedure as well as the cat experiments will be described in the appropriate chapters.

Animals.

Dogs with vagotomized gastric pouches have been used for the investigations. The operations were carried out by Heidenhain's method (*fig. 1*). For technique see e. g. Le Play (1912). To avoid digestion

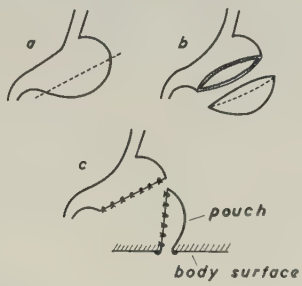

Fig. 1. *The Heidenhain pouch.* A schematic view of the operation.

of the surroundings of the "mouth" of the pouch, precautions were taken to prevent the gastric juice from flowing over the skin. This was achieved by using a Pezzer catheter (fig. 27, page 63) introduced through the opening of the pouch and allowed to remain there. The dogs usually very quickly became accustomed to this procedure which proved very effective in preventing digestion of the skin. It also had the advantage that a special measuring cylinder could be connected to this permanent rubber tube when desired.

The experiments were carried out on *fasting dogs. All possible care was taken to avoid psychic stimulation of the secretion.*

Before starting an experiment the pouch was usually washed out with warm saline, generally 20 ml. All the dogs were healthy and did not show any sign of discomfort during the performance of the present work.

For the present investigations 7 dogs were used. 3 of these in particular (dog S, R and B) were used for long periods (3—1 years). Two died from a prolapse of the pouch, two by an accident and one from distemper. Dogs S and B are still alive.

Stimulation of the gastric glands.

Histamine dihydrochloride[1] was used as a stimulating agent.

Popielski (1920), Keeton, Koch & Luckhardt (1920) and Rothlin & Gundlach (1921) discovered that histamine (β-imidazolylethylamine) had a powerful stimulating effect on the gastric glands when given subcutaneously, but failed to stimulate the glands when injected intravenously in a massive dose. Ivy & Javois (1924) and Gutowski (1924 a) when giving histamine intravenously over a period of 30 or 50 minutes, succeeded in getting a copious flow of gastric juice. They realized that not only the amount of histamine given, but also the *time* was of importance.

Teorell (1932, 1933) was able to show mathematically that in Popielski's experiments the histamine was probably destroyed in the blood before causing any stimulation of the gastric glands. He proved that *a prolonged histamine concentration in the blood must be maintained if a stimulating effect is to be obtained.*

In order to balance the rate of the histamine destruction an appropriate amount of the drug must be administered by a *continuous intravenous injection.* The kinetics of the distribution of a drug administered in this way has been discussed by Widmark (1919), Widmark & Tandberg (1924) and by Teorell (1937 a, b). This stimulation technique for the gastric glands has been used by only a few authors: Ivy & Javois (1924), Gutowski (1924 a), Teorell (1933), Engeström (1935), Emmelin, Kahlson & Wicksell (1941), Björkman, Nordén & Uvnäs (1943), Emmelin (1945), McElin & Horton (1946) and Linde, Teorell & Öbrink (1947).

Technique of injection.

In some experiments the histamine was given *subcutaneously* but the intravenous route was generally used.

The *continuous intravenous injection* was performed by using a *motor driven syringe.* At the beginning, an apparatus described by Lindgren (1943) was used, but later on a new device, which has proved very suitable, was adopted.[2]

[1] In this paper all figures are expressed in terms of *histamine-dihydrochloride.* To transform these to histamine base or histamine diphosphate the figures must be multiplied by 0.60 and 1.67 respectively.

[2] The technical work and the details of construction were executed by Mr. S. Liliedahl.

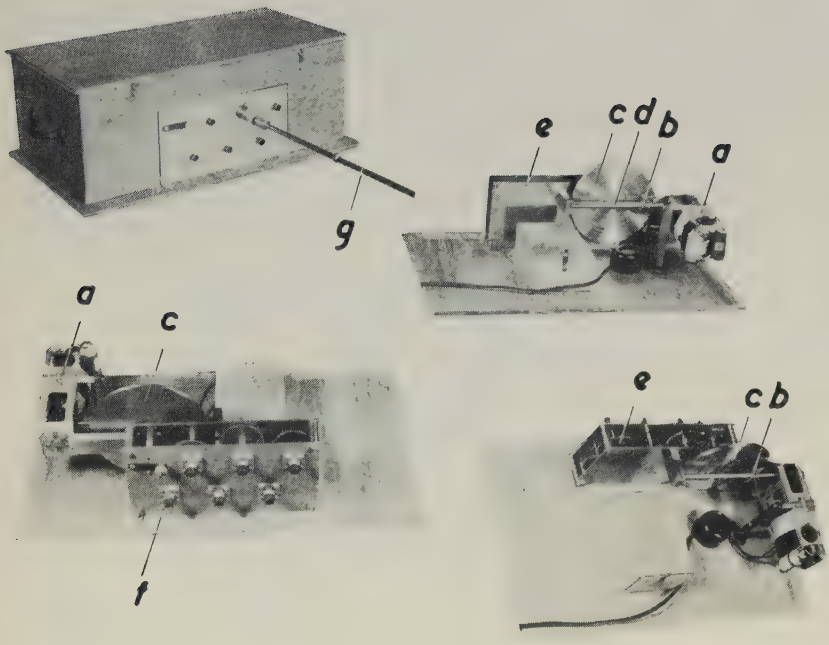

Fig. 2. *The injection apparatus* (*Motor part*). *a* is the electric motor, which drives the small wheel *b*. This acts on the large wheel *c*. *b* can be moved along the axle *d*. The speed is lowered in the gear box *e* 5 times between each connection *f* for the spindle *g*, which leads to the syringe holder in fig. 3.

As such an apparatus may often be useful in other laboratories a short description will be given here.

The injection apparatus.

The motor part (*fig. 2*).

The electric motor (a) is an ordinary gramophone motor (but of course any motor that has a very constant speed may be used). The motor drives a small wheel (b) 28 mm in diameter, the peripheral part of which is made of rubber and pressed against a larger wheel (c) 150 mm in diameter. The axles of the two wheels are at right angles to each other. By adjusting the small wheel nearer to or further from the center of the big one, the speed of the latter may be made greater or less. For this purpose the small wheel can be moved along an axle (d) graduated from 1 to 6, where 1 corresponds to the periphery of the big wheel and 6 to the center. It is not

necessary nor convenient to place the rubber-wheel nearer the center than at 5. Thus by moving the small wheel from 1 to 5, the speed can be increased 5 times.

From the large wheel an axle leads to a gear box (e). This lowers the rate in steps of 5 times. The lowest rate is 5^{-5} times the rate of the big wheel. By altering the position of the little wheel and by using different connections (f) of the gear box the speed of movement derived

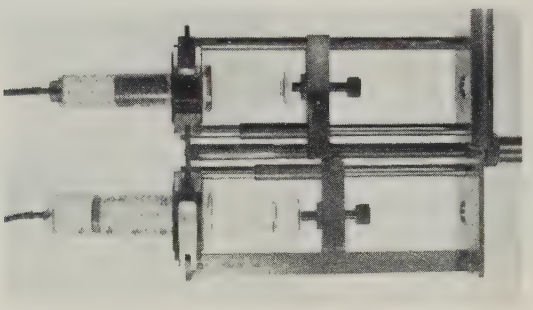

Fig. 3. *The injection apparatus* (*Syringe holder*). This is only a modification of the one constructed by LINDGREN (1943).

from the machine may be changed by a factor as much as 5^6. The lowest speed is about 0.4 turns/hour and the highest about 100 turns/minute. From the gear box a connecting spindle (g) leads to the syringe holder.

The syringe holder (*fig. 3*).

The principal part of this is a screw, which is driven by the spindle. The screw drives two cross-pieces which are in contact with the syringes. The mechanism of this part is the same as described by LINDGREN (1943). The only difference is that two syringes can be driven at the same time instead of one. If the height of the screw-thread is 1 mm, that distance corresponds to one turn of the spindle.

In order to determine the position of the little wheel and the spindle, the diagram in *fig. 4* can be used. If a particular speed for the syringe is desired this must be transformed into turns/minute of the spindle. 100 times the reciprocal of that value may be looked up in the diagram. The column in which this quotient is found indicates the connection point for the spindle and the horizontal intercept to the crossing point between a horizontal line from the number and the oblique line in the diagram indicates the position of the small wheel (graduations on the axle).

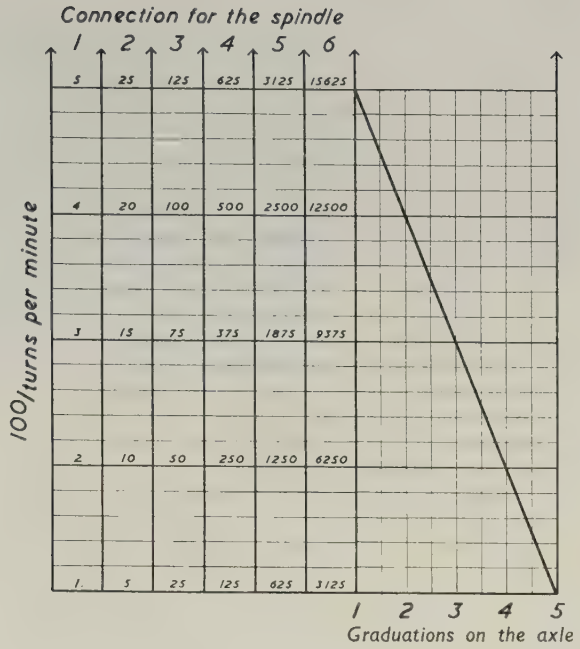

Fig. 4. *Diagram for the use of the injection apparatus.* For description see text! (*Example:* The injection rate wanted needs 2 turns per minute by the screw of the syringe holder. 100/turns per minute — the ordinate in the figure — is 100/2 = 50. This number is found in column 3, which means that the spindle must be connected to the third connection. The horizontal from 50 crosses the oblique line at the graduation 4, which indicates where the small wheel shall be placed on the large one.)

The contents of the syringe are pressed through a narrow rubber tube to a needle of the shape in *fig. 5*. By using this type of needle the rubber tube can be connected to the one mouthpiece, while blood can be obtained through the other one, thus indicating a perfect position in the vein. The needle is fixed with adhesive tape.

Method of collecting the gastric juice.

A *special measure* is seen in *fig. 6*. It consists of a measuring cylinder supplied with a stopcock with a very wide hole. This makes it possible to collect the juice for a desired time and to empty the measure instantaneously even if the content should have a high viscosity. A suction pump can be connected to a side-pipe, if desired. A negative pressure of 30 cm of water is then convenient. The tube, which is graduated in 1/5 of a ml, is joined to the Pezzer catheter during the experiment.

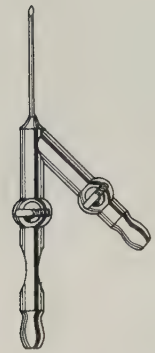

Fig. 5. *The injection needle.*

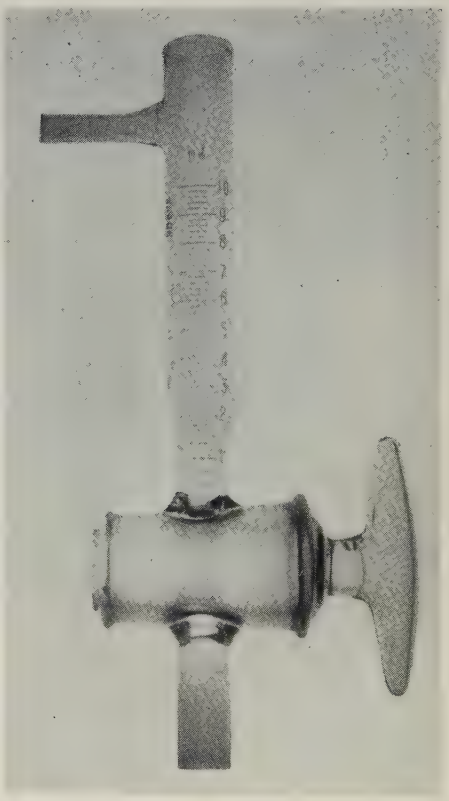

Fig. 6. *The measuring cylinder* for collecting the gastric juice.

By the technique described in this chapter it is found possible to record the secretion volumes at very short intervals. *2 minutes was usually the time used for determining the secretion rates* whereas *samples for analyses were taken every 10 minutes.*

CHAPTER 3.

Methods of Analysis.

All the analytical procedures are collected in this chapter. They are described in the following order and include the determinations of

1. *pH*
2. *acidity*
3. *chloride*
4. *histamine*
5. *pepsin*
6. *nitrogen*
7. *reducing power*
8. *calcium*
9. *bicarbonate*
10. *phenol red*
11. *neutral red*

The *statistical errors of the estimations* are given in connection with the description of the methods.

They are expressed as the errors of *single determinations* and were calculated as the standard deviation from series of 20 separate determinations on identical objects. If "blanks" and/or "standards" are included in the analytical procedure, their errors are also considered.

In some methods (2, 3 and 8) the result (x) was calculated from a "*standard*" (st) with its error σ_{st} and a "*sample*" (sa) with its error σ_{sa} as

$$x = \frac{sa}{st} \cdot C \tag{1}$$

(C is a constant).

The error of a single determination was then calculated as

$$\sigma_x = \pm C \cdot \frac{\sqrt{st^2 \sigma_{sa}^2 + sa^2 \sigma_{st}^2}}{st^2} \tag{2}$$

In some methods (6, 7 and 9) the result (x) was calculated from a "*blank*" (bl) with its error σ_{bl} and a "*sample*" as

$$x = (bl - sa) \cdot C \tag{3}$$

The error of a single determination was then calculated as

$$\sigma_x = \pm C \cdot \sqrt{\sigma_{bl}^2 + \sigma_{sa}^2} \tag{4}$$

1. *pH determinations.*

pH was determined by aid of a glass electrode arrangement. Accuracy of reading $\pm$ 0.05 pH units.

2. *Acidity determinations.*

a) Total acidity:

The volumes available for the acidity determinations varied from some mls down to the order of 0.1 ml. A microtitration on 0.1 ml juice mixed up with 4 ml H_2O was therefore performed with 0.01 N NaOH using *brom-thymol-blue* as an indicator (pH-range 6.0—7.6). The strength of the NaOH solution was checked every day using carefully controlled 0.1 N HCl (standard). The same pipette was used for the standard and the samples. Using this procedure errors due to the pipette, indicator, and CO_2 admixture were compensated. The neutralization of NaOH by 4 ml H_2O was determined as a blank. (This was in most cases in the order of 0.00—0.01 ml. The error of the blank determination will have an insignificant influence on the error of the method.)

b) Free acidity:

Free acidity was determined either by an electrometric titration (pH 3.5) or by the use of Töpfer's indicator (di-methyl-amino-azo-benzene, endpoint pH 3.5). The volume used for these titrations was 1.0—5.0 ml.

With the histamine technique used in the present paper the difference between the free and the total acidity was very small. For this and other reasons (cf. page 52) only the total acidity values are discussed in the following.

The error of the total acidity determinations (valid for the total range in this paper): = ± 2 mN[1].

3. *Chloride determinations.*

a) in gastric juice:

The chloride titrations were performed on the same samples as were used for the total acidity determinations.

2 ml 0.1 N H_2SO_4 were added and then the samples were titrated electrometrically with 0.005 N $AgNO_3$.

b) in blood plasma:

Heparinised blood was centrifuged. 0.1 ml plasma + 4 ml H_2O + 2 ml 0.1 N H_2SO_4 were titrated in the same way as the gastric chlorides. The proteins do not affect the result.

Standard and blank titrations were performed as in the acidity determinations.

The error of the method varied from time to time according to the conditions of the measuring instruments. Under bad conditions it was ± 6 mN but all the data referred to in this paper have the smaller error given below.

The error of the chloride determinations (valid for the total range in this paper): ± 2 mN[1].

4. *Histamine determinations.*

The method described by Code (1937) was used. The determinations were performed on 4—5 ml plasma from heparinised blood. The concentration-action curve of histamine on the guinea pig's small intestine was determined and is seen in *fig. 7* — cf. Emmelin, Kahlson & Wicksell (1941). Our curve, like that of the authors referred to, demonstrates the necessity of carrying out the investigations with concentrations of histamine which cause only a submaximal contraction (the rising part of the curve). All histamine values are expressed as histamine dihydrochloride (cf. page 10).

The error of the histamine determinations in blood plasma: ± 3.7 μg/100 ml = 5 % of the mean value.

5. *Pepsin determinations.*

Pepsin determinations were carried out according to the method described by Riggs & Stadie (1943). This method consists of a turbidimetric analysis of a protein solution, which was digested for 5 minutes. The digestive power is expressed in mg protein digested per ml gastric juice during 1 minute.

The error of the pepsin determinations: ± 0.005 mg protein/ml/min. = 1.8 % of the mean value.

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[1] mN = millinormality = milliequivalents per liter.

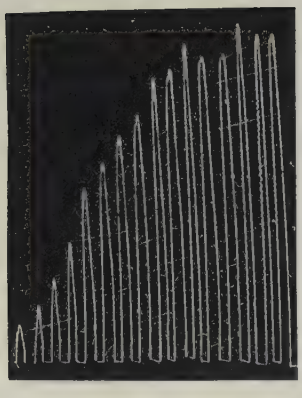


Fig. 7. *Concentration-action curve* of histamine action on a guinea pig's small intestine. The contractions correspond to the histamine-doses: 0.005, 0.010, 0.015, 0.020 etc. µg histamine dihydrochloride per ml bath solution. The volume of the bath = 10 ml.

6. *Nitrogen determinations.*

1.0 ml gastric juice was combusted with 1 ml concentrated H_2SO_4 for 5 hours. 2—3 drops of H_2O_2 were added and the combustion continued another 2 hours. The determinations were then performed by the method of Teorell (1928).

The error of the nitrogen determinations (valid for the total range in this paper): ± 4.0 mg N/100 ml.

7. *Determinations of reducing power.*

2 ml of gastric juice + 5 ml of a 10 per cent HCl were heated in a boiling water bath for 30 minutes. After cooling they were neutralized with 30 per cent NaOH. The reducing power of the remaining volume and of another 2 ml juice were then determined — cf. Ihre (1938).

This was done by the method of Schales & Schales (1945) with the modification that a sodium-cyanide-carbonate solution was used instead of a phosphate buffer solution. This modification which was mentioned by Schales and Schales was adopted from the paper of Folin & Malmros (1929).

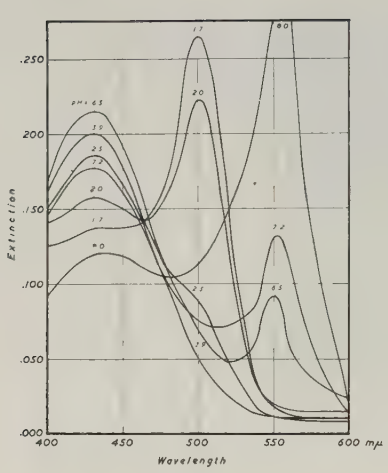
The difference in the reducing power of the hydrolyzed and the non-hydrolyzed sample was compared with that of known solutions of glucose and expressed in mg glucose per 100 ml.

The error of the reducing power determinations (valid for the total range in this paper): ± 8 mg/100 ml.

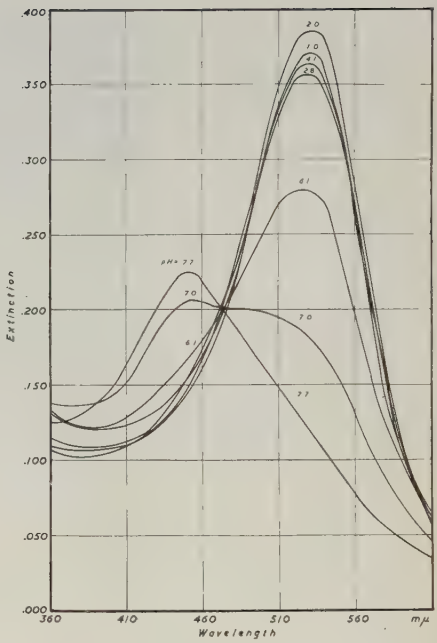
8. *Calcium determinations.*

The oxidimetric method with ceric sulphate as described by Nielsen (1941, p. 190) was used.

The error of the calcium determinations (valid for the total range in this paper): ± 0.45 mg/100 ml.



a



b

Fig. 8. *Absorption curves of phenol red (a) and neutral red (b) at different pH:s.*

9. *Bicarbonate determinations.*

The bicarbonate concentration in glycine solutions was determined according to the method of West, Christensen & Rinehart (1940).

The error of the bicarbonate determinations (valid for the total range in in this paper): ± 2.8 mN.

10. *Phenol red determinations.*

The concentration of phenol red was determined in the estimations of the size of the "*lake*," which is a residual volume of the contents in the gastric pouch (cf. page 63).

Some exact volume, *a* ml, of phenol red was instilled into the pouch cavity and mixed up with the "lake" contents as far as possible. The phenol red solution was then allowed to leave the pouch.

The colour of the dye was determined before (C_a) and after (C_x) the instillation. The size of the "lake" (x) was then easily found from

$$x = \frac{a\,(C_a - C_x)}{C_x} \qquad (5)$$

This gave a minimal value of the "lake" because there was no guarantee that a complete mixing had occurred.

In order not to let changes in pH influence the colour determinations in a Coleman electrophotometer, these were carried out at the *isosbestic point* (wave length 463 mμ). This was found by determining the absorption curves of the same concentration of phenol red at several different pH:s in a Beckman spectrophotometer. The absorption curves are seen in *fig. 8 a.*

The error of the phenol red determinations: ± 0.6 % of the mean value.

11. *Neutral red (N. R.) determinations.*

N. R., like phenol red, was determined in a Coleman electrophotometer at the *isosbestic point* (wave length 472 mμ).

The absorption curves from which the isosbestic point is obtained are seen in *fig. 8 b.*

The error of the neutral red determinations: ± 0.6 % of the mean value.

CHAPTER 4

The Histamine Distribution in the Body.

Theoretical considerations.

Introduction.

It is well known that the concentration in blood and tissue of a drug given subcutaneously or intravenously will follow different *time-concentration curves.* It is easy to understand that a very high concentration in the blood will occur immediately after a rapid intravenous injection, whereas a subcutaneous injection will cause a slower increase. The *kinetics* (i. e. the time relations of the drug concentration) will depend on the drug itself and the possibility of its passing from one phase to another and the forces causing this passage. Reactions of the drug, such as combination or destruction, will also influence the concentration of the active drug. In the case of an intravenous injection the *injection rate will be of importance.*

Very little study has been devoted to this problem. Teorell (1937 a, b) has tried to develop a general mathematical view of the problem. In his papers a short review of the earlier literature in this field is given. It is therefore unnecessary to repeat it here. It is, however, necessary to give a short summary of Teorell's results, in order to make it possible to discuss some problems in our own work.

Teorell's work.

The validity of *the mathematical formulae given is limited to electrically uncharged substances,* i. e. strong electrolytes do not behave in the same manner as undissociated molecules because they are also influenced by electrical potential gradients. If the concentration gradient is the only driving force, the amount passing a boundary will follow Fick's law for unidimensional molecular diffusion:

$$Amount = diffusion\ coeff. \times conc.\ gradient \times surface \times time.$$

If the diffusion coeff. and surface are taken together in a "*permeability coefficient*" as k_n' the formula may be rewritten.

$$Amount\ across\ boundary\ in\ the\ time\ unit = \\ = permeability\ coeff. \times concentration\ difference.$$

A concentration is, however, the amount divided by the appropriate volume, and so we may take the permeability coefficient together with the distribution volume, which can be considered as constant, and substitute them with a "*velocity constant*" k_n. The temperature is supposed to be constant.

The velocity constants involved are:

k_1 for the diffusion depot → blood (extravascular administration only)
k_2 » » » blood → tissue
k_3 » » » tissue → blood
k_4 » » » blood → kidneys or lungs (or blood inactivation)
k_5 » » tissue inactivation.

A. *The subcutaneous case.*

If x, y and z denotes the amount of the drug in *depot, blood* and *tissue* respectively, and N_0 = the initial dose given to the depot, the following differential equations may be derived:

for the blood:

$$\frac{dy}{dt} = k_1 x - k_4 y - (k_2 y - k_3 z) \tag{6}$$

for the tissue:

$$\frac{dz}{dt} = (k_2 y - k_3 z) - k_5 z \tag{7}$$

By combining the two equations it is possible to solve for y and z:

$$y = C_1 e^{m_1 t} + C_2 e^{m_2 t} + S e^{-k_1 t} \tag{8}$$

$$z = R_1 e^{m_1 t} + R_2 e^{m_2 t} + H e^{-k_1 t} \tag{9}$$

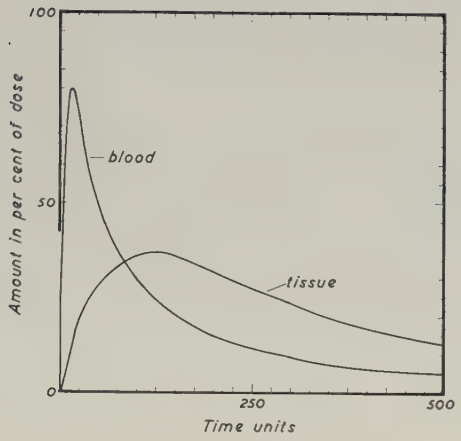


Fig. 9. *The distribution of a drug injected subcutaneously.* See eqs (8) and (9). [$k_1 = 0.2$; $k_2 = 0.01$; $k_3 = 0.005$; $k_4 = 0.005$; $k_5 = 0.002$.] — After TEORELL (1937 a).

where the new symbols C_1, C_2, S, R_1, R_2, H, m_1 and m_2 are all constants and functions of the velocity constants k_1—k_5 and the dose N_0.

The relations (8) and (9) are seen in *fig. 9*, where the amounts in per cent of the initial dose N_0 are plotted against time.

B. *The continuous intravenous case.*

In the case of a *continuous intravenous injection* the rate of "resorption" will be substituted by the injection rate, denoted by r. Thus in equation (6), $k_1 x$ may be substituted by r. The rest of the system remains unchanged. The final mathematical solution can then be written:

$$\boxed{y = r \cdot [A_1 e^{m_1 t} + A_2 e^{m_2 t} + I]} \tag{10}$$

and

$$\boxed{z = r \cdot [B_1 e^{m_1 t} + B_2 e^{m_2 t} + J]} \tag{11}$$

where A_1, A_2, I, B_1, B_2 and J are constants and functions of the velocity constants k_2—k_5. The rest of the symbols retain their previous significance.

Fig. 10 gives the graphical representation of the equations (10) and (11). It must be noted that both the blood and tissue curves approach a constant value, which can be mathematically expressed by putting time $t = \infty$.

Then (10) and (11) are reduced to:

$$y_\infty = r \cdot \frac{k_3 + k_5}{q} \tag{12}$$

and

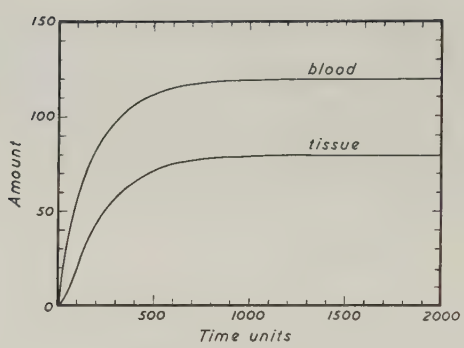


Fig. 10. *The distribution of a drug after continuous intravenous injection.* See eqs (10) and (11). [$r = 1$; $k_2 = 0.01$; $k_4 = 0.005$; $k_5 = 0.005$.] — After TEORELL (1937 b).

$$z_\infty = r \cdot \frac{k_2}{q} \tag{13}$$

where $q = k_2 k_5 + k_3 k_4 + k_4 k_5$.

The above is a short review of those parts of the work of TEORELL, which have an application to the present paper. Starting from these equations some other relations can be investigated:

The author's calculations.

If the interest is directed for a moment to the *total amount* of a drug accumulated in the blood and tissue after a subcutaneous injection during the whole period of an experiment, the following will be found:

The *total amount in the blood* is represented by the area included in the curve given in eq. (8). This area is

$$Y = \int_{t=0}^{t=\infty} y \, dt \tag{14}$$

where y has the same significance as in eq. (8). Integration of (14) gives

$$Y = \frac{k_3 + k_5}{q} \cdot N_0 \tag{15}$$

In an analogous way the *total amount in the tissue* will be found by integrating the eq. (9) and the result is

$$Z = \frac{k_2}{q} \cdot N_0 \tag{16}$$

The first point of interest is that k_1 has disappeared from these formulae, i. e. *after a subcutaneous injection the total amount of a drug*

found in blood or tissue is independent of kind or rate of resorption. It depends only upon the dose given in the depot.

On the contrary the *maximal value* obtained in blood or tissue during an experiment after a subcutaneous injection will not be independent of k_1, as may easily be shown. The general expressions, however, are too complicated to be solved here. (TEORELL has proved it for blood in a special case ($k_2 = k_3 = k_5 = 0$).)

The integrations for (15) and (16) were made from $t = 0$ to $t = \infty$. In practice the result of integration will be just the same if it is made between $t = 0$ and $t =$ some short measurable time (e. g. 1—3 hours) when we are dealing with drugs, that are quickly eliminated or destroyed as histamine.

The second point of interest is the similarity that exists between the eqs (12), (13) and (15), (16).

The consequences of these expressions will be discussed below (chapter 7, page 37).

Experimental data.

Earlier experiments.

There have been many observations after continuous intravenous injections of substances which support the theoretical considerations given above. The concentration of any substance injected in this way will undoubtedly approach a constant level. Both non-electrolytes and electrolytes may be taken as examples. See WIDMARK (1919) for acetone and WILHELMJ & MOSKOWITZ (1932) for uric acid.

The behaviour of histamine itself has also been investigated — EMMELIN, KAHLSON & WICKSELL (1941), EMMELIN (1945). The main thing is, however, to prove that the steady state of concentration is proportional to the injection rate (according to eq. 12 and 13). This has not been done to the author's knowledge. The studies of EMMELIN & KAHLSON (1944) have been restricted to correlating the injection rate and the concentration of histamine in the gastric juice, and their results corroborate the theoretical propositions.

The author's experiments.

The method used for the histamine determinations was the one proposed by CODE (1937). (Cf. page 16.)

Analyses were made in the venous blood from a leg before the start of the experiment, "*base value*," and when the injection of histamine had caused a steady state of the secretion rate, "*steady state value.*"

The increment caused by the histamine injection is the "*steady state value*" *minus the* "*base value.*"

In some series of experiments the analyses were performed on *whole blood* but gave no regular covariation of the histamine increment and the injection rate of histamine.

In the experiments described below the *plasma* was analyzed. The injection rates were changed at random. The rates of injection were usually less than 8 ml/hour.

In every experiment there was a considerable "base value," which did not cause any gastric secretion. If the method of analysis was specific for histamine it means that histamine was present in the blood plasma in an inactive form. This question will be discussed below.

The main question is to determine the possible covariation between the rate of injection of histamine and the steady state increment. *Fig. 11* shows this relation (dog S), and the experimental values are correlated to the eq. (12). The coefficient of correlation is 0.94. The other statistical data are seen in the legend to the figure.

The filled circles in the figure denote the corresponding secretion rates. They indicate that most of the analyses were performed during *supramaximal* stimulation. This is essential as it shows that the linear covariation between the rate of injection of histamine and its concentration in plasma is independent of the physiological action on the gastric mucosa. (The relations between this action and the histamine concentration will be discussed in detail in chapter 6.)

The results would permit the use of eq. (12) in the following discussions. If eq. (13) were valid it would be even more useful, but this has not been established. The present results and those of Emmelin & Kahlson (1944), however, make this relation seem most probable, i. e. *the concentration of active histamine in the tissue is directly proportional to the injection rate of histamine.*

General reactions of the animals after histamine administration.

The administration of large doses of histamine (more than 3 mg/kg/hour) may cause a severe shock — Dale & Laidlaw (1919) — and a considerable decrease of the blood pressure — McCarrel & Drinker (1941). In the present

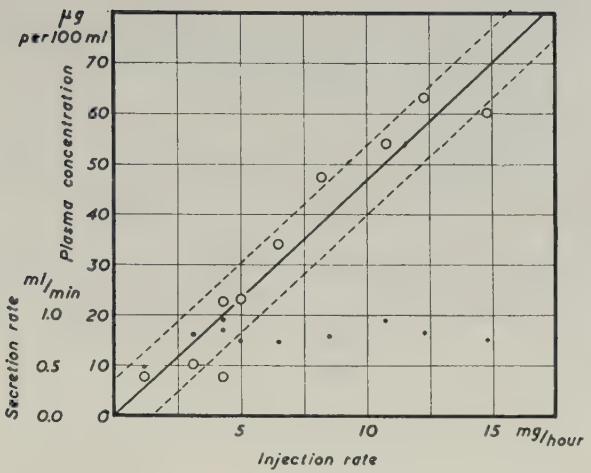


Fig. 11. *The relation between the injection rate of histamine and the steady state values of the histamine concentration in plasma.* The filled circles are the corresponding secretion rates. (The regression coefficient = 4.68; the standard error of estimate = ± 7.7; the coefficient of correlation = 0.94.)

experiments the largest dose has been about 0.6 mg/kg/hour and mostly not more than 0.25 mg/kg/hour were used. It is thought that these doses do not cause any persistent decrease in the blood pressure. It was only with the very highest injection rates that a slight dyspnoea was observed, but otherwise no undesired reaction could be noticed.

In a few experiments with narcotized cats (chloralose-urethane) the results of Giraud-Costa & Gayral (1940) and Emmelin (1945) have been confirmed, namely that the injection of small doses of histamine may cause an immediate decrease in the blood pressure which returns to its original level in a few minutes.

In what form does histamine exist in the plasma?

This question has been widely discussed by several authors. Tarras-Wahlberg (1936) considered that histamine occurred in the free form under special conditions. Kaiser (1939) was of the opinion that histamine was bound to protein and inactive, which was also stated by Rocha e Silva (1942, 1943, 1944). Emmelin (1945), from several kinds of experiments, concluded that histamine exists in plasma in a physiologically active form, or as an inactive compound from which histamine is extremely easily liberated. His results were criticized by Colldahl, Holmberg & Laurell (1946).

In the present experiments the dogs showed a high concentration in the plasma of substances which acted upon the guinea pig gut, even when no gastric secretion could be obtained. This activity may have been due to histamine, but there was no stimulation of the gastric glands. The reason might be a) that histamine occurred in a physiologically inactive form or *b*) that the gastric glands were in some way adapted to the actual histamine concentration and started the secretion only for an *increase* in the plasma histamine level.

In order to determine whether histamine alone was responsible for the gut contractions, the following experiments were performed:

Some *antihistamine* preparations were added to the Tyrode's solution in the intestinal bath. NILZÉN (1947) gives some concentrations of such drugs which he found necessary to inhibit the contractions. By using the same concentrations of *Antasten*[1] (Ciba) as Nilzén — 1.0 μg/ml — a pure histamine solution was rendered ineffective and the plasma extract was deprived of about 2/3 of its activity.

After a very large dose of *Lergitin*[2] (Recip) — 1 mg/ml — no contraction could be initiated either by pure histamine or by the plasma extract (the intestine did, however, still react on addition of acetycholine).

These findings make it seem probable (but do not prove) that the "base values" of the plasma extracts were histamine.

It seems very difficult to disprove the cross-circulation experiments performed by EMMELIN (1945), which proved that histamine existed in an active form. On the other hand COLLDAHL, HOLMBERG & LAURELL (1946) have disproved the existence of any adaptation. In the present experiments the "base value" was not constant and would sometimes show a marked decrease from day to day. This may also be in opposition to an adaptation theory.

It seems, on these findings, *probable* that histamine may occur in an inactive form in the blood.

CHAPTER 5.

Time-secretion Relation after Continuous Intravenous Injection of Histamine.

Effect of constant injection rates.

From chapter 4 the relation between the injection rate of histamine and the blood concentration is known. It is found that, in accordance with the theoretical considerations, *the blood concentrations will approach a steady state value* which is proportional to the injection rate. When the concentration in the blood is constant that in the tissues must also be considered to be constant as a constant blood concentration indicates a dynamic equilibrium between blood and tissue.

Considering the tissue concentration of histamine as following the theoretical curve in fig. 10, it will be of interest to know how the histamine effect in the gastric mucosa will proceed. *Fig. 12* shows a typical experiment, which gives an answer to the question. The injection of

[1] 2-phenylbenzylaminomethylimidazolin hydrochloride.

[2] Dimethylbenzylphenylethylendiamin hydrochloride.

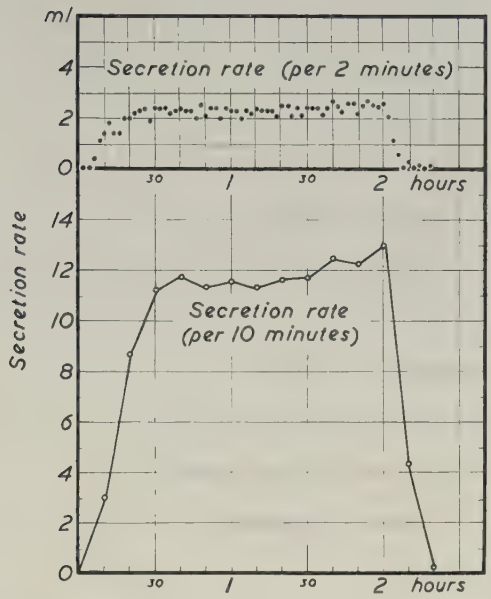


Fig. 12. *A typical experiment showing gastric secretion after continuous intravenous injection of histamine.* The injection is started at 0 hours and continued at a constant rate till 2^{00} hours when it is stopped. The upper part of the diagram shows the secretion rate expressed in ml/2 min; the lower part the corresponding ml/10 min.

histamine is started at time 0 and continued at a constant rate. After about 6 minutes the secretion starts and rises to a constant value which is reached in about 30 minutes. This means that there is a time lag of about 30 minutes before the rate of secretion reaches its final value, a fact which may be due to there being a similar time lag before the final steady value of the histamine concentration in the tissues is reached.

The secretion rates are expressed in ml/10 minutes (the lower part of the figure) and ml/2 minutes (the upper part). Cf. the "Procedure" chapter (page 14).

Several experiments have shown that *a constant value of the secretion rate can be maintained* for at least 8 hours. A sign of fatigue has been noticed only on one dog at high injection rates.

The general appearance of the curve resembles that of the time-concentration curve of a drug illustrating the theoretical considerations in chapter 4 (eq. 11 and fig. 10). Notice the time lag, which makes the curve concave upwards from the start at its origin. It is

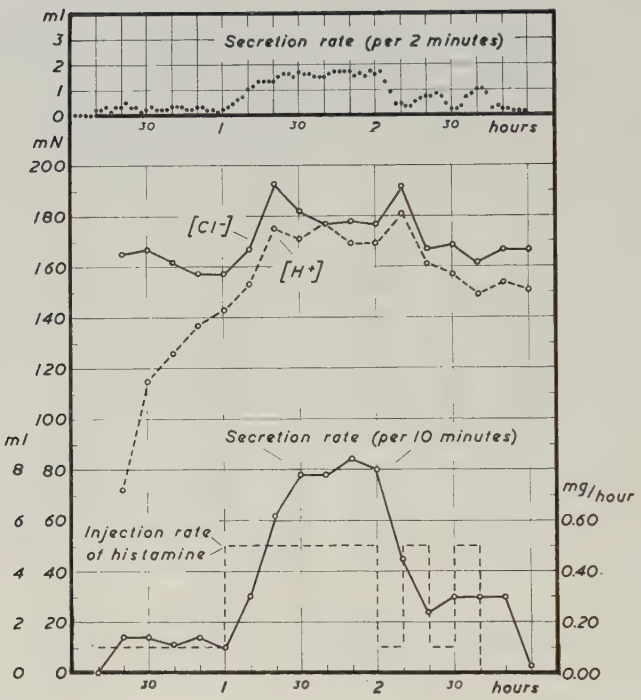


Fig. 13. *The apparent disagreement between the injection rate of histamine and the secretion rate.* This disagreement only appears when the amounts secreted over periods of 10 minutes are considered and is not apparent on consideration of the amounts secreted over periods of 2 minutes. (See text.)

not unexpected to find such a time relation, as *the effect of histamine in inducing secretion must be related to its concentration in the tissue* (*mucosa*). Cf. TEORELL (1937, c).

Effect of changing the injection rate of histamine.

The above mentioned results have the following interesting consequences. As the injected histamine has to pass through the blood stream before it enters the gastric mucosa, a 30 minutes time lag will occur between a *change* in the injection rate and a new steady state value of the gastric secretory rate.

This may sometimes cause surprising results, which have been seen in experiments of which a typical one is given in *fig. 13.* When a sufficient time has elapsed after starting the injection, and the secretory rate (s. r.) has become constant, the injection rate (i. r.) is sud-

denly increased 5 times (at 1^{00} hours). After about 30 minutes a new constant secretion rate is obtained. At 2^{00} hours the i. r. is lowered to its original value but after 10 minutes the rapid rate of injection is resumed. After another 10 minutes the slow rate is repeated; the rate of injection thus alters every 10 minutes. If we look at the amount secreted *every 10 minutes* in fig. 13 we see the remarkable fact, that

the 1st lowering of the i. r. apparently causes a decrease in the s. r. (2^{00} hrs),
» 2nd *increasing* of the i. r. apparently causes a *decrease* in the s. r. (2^{10} hrs),
» 2nd *lowering* of the i. r. apparently causes an *increase* in the s. r. (2^{20} hrs),
» 3rd *increasing* of the i. r. apparently causes a *decrease* in the s. r. (2^{30} hrs),
» 3rd lowering of the i. r. apparently causes no change in the s. r. (2^{40} hrs).

These observations seem to support the idea that there is *no* close relation between the i. r. and s. r.

Such a deduction is, however, false and can easily be explained by studying the secretory rate *every 2 minutes*. These observations are shown on the top of fig. 13. They show a very good correlation between the i. r. and the s. r. The explanation of the paradoxical observations obtained by making a reading every 10 minutes is, that the *time lag retards the reaching of a steady state value of secretion*. The secretion rate will change immediately (increase or decrease) with the changes in the injection rate, but a steady state value is not reached in 10 minutes (before the next alteration). This makes it possible for the total amount of juice (i. e. the integrated area) to vary independently of the rate of secretion (and injection). It should be stressed that this independence is only an apparent one due to the special conditions of collection.

Example: During 10 minutes an increasing secretion rate may yield $1+2+3+4+5=15$ ml of juice, while a decreasing rate may give $8+7+6+5+4=30$ ml. If we do not consider the *changes* in the secretion rate during a stimulation period but only the total amount secreted, we may get an erroneous view of the process.

This has the practical consequences that *a secretion rate ought to be studied at short intervals if it is not uniform.*

The relation between stimulation and secretion is further discussed in the next chapter.

CHAPTER 6.

Relation between Histamine Concentration and Rate of Secretion. Concentration-action Curves.

Theoretical considerations.

Histamine acts directly upon the peripheral structure of the gastric mucosa. It has been proved many times, that no nerve supply by the vagus to the stomach is required for its response to histamine — Popielski (1920), Keeton, Luckhardt & Koch (1920), Koskowski (1922) and Ivy & Javois (1924). Even a transplanted piece of mucosa secretes hydrochloric acid after stimulation with histamine — Ivy & Farrell (1925), Klein & Arnheim (1932) and Grossman & Ivy (1946). *It may therefore be expected, that a close relation will be found between the histamine content of the mucosa and its secretion rate.* — cf. Teorell (1937 c).

Lips, Verschure & Strengers (1947) could not find any covariation between histamine concentration in the blood and gastric acidity, but this may be due to the specific experimental procedure employed.

The mechanism in which histamine takes an active part is unknown. We can, however, make a rough model of the system which may incorporate the mechanism while remaining ignorant of its details.

Define the secretory process as the "*producing flow*," which is converted into hydrochloric acid by means of histamine.[1] Consider the maximal response of the mucosa towards histamine to be a ml/min., which will be secreted when all of the "producing flow" is converted into hydrochloric acid.

At a certain concentration of histamine in the mucosa, z,[2] some part — or the whole of it — is causing secretion of gastric acid.[3]

We may call the *rate* of this contribution of histamine or better flux of histamine to the secretory process ϕz. This ϕz is certainly proportional to z and consequently to the injection rate of histamine r. (Cf. eq. (13).)

[1] This "producing flow" may be the production of a real precursor to the HCl or of different chemical compounds which take part in the acid formation.

[2] Cf. eq. (13).

[3] Histamine may be consumed during the secretory process or act in a catalytic way. The following argument will be the same in both cases.

Thus we may put

$$\phi z = k_1 r \tag{17}$$

ϕz is converting the "producing flow" into hydrochloric acid at a rate of v ml/min.

If ϕz is increased by $d\phi z$ this increment may react with the "producing flow" still left, $(a - v)$, and thus cause an acceleration of the secretion, dv. Following the law of mass action dv will be proportional to the product of $d\phi z$ and $(a - v)$ or

$$dv = K(a - v)\, d\phi z \tag{18}$$

where K is a constant.

Integration of this equation gives

$$v = a(1 - e^{-K\phi z}) \tag{19}$$

or by substitution of (17) and putting $K k_1 = k$

$$\boxed{v = a(1 - e^{-kr})} \tag{20}$$

This is a well known mathematical expression. It is graphically represented by a curve passing through the origin and rising exponentially to an asymptotic value a. — cf. Öbrink (1947 b).

Experimental data.

In order to get satisfactory experimental results in this field, the adoption of the method of continuous intravenous injection affords a great help in getting constant and easily determined secretory rates. In the present experiments the constancy has been observed for at least fifteen 2-minute periods and the mean of the secretory rate has been calculated. The values are expressed in ml/min (not in ml/2 minutes).

From the experiments on one dog (19.7 kg) the injection rates of histamine and the corresponding rates of secretion are given in *Table 1.* The third column in this table shows that the injection rates have been changed at random. The concentration of histamine injected has also been varied as is seen in the second column.

Plotting the secretion rates as the ordinate in a coordinate system with the rates of injection of histamine as the abscissa we get the *fig. 14.*

How does eq. (20) fit these points? That is a matter of correlation analysis.

Table 1. *The relation between injection rate of histamine and secretion rate of gastric juice.*

Date	Conc. of histamine injected mg/ml	Rate of injection mg/hour	Rate of secretion ml/min.
31/1	0.50	3.40	1.39
5/2	0.75	2.85	1.06
9/2	0.75	2.85	0.96
11/2	0.25	0.95	0.54
11/2	0.25	4.80	1.18
12/2	0.10	0.40	0.21
12/2	0.10	1.90	0.98
13/2	0.10	0.40	0.30
13/2	0.10	0.68	0.41
14/2	0.25	0.95	0.43
14/2	0.25	1.30	0.67
15/2	1.00	3.80	1.03
15/2	1.00	5.20	1.25
20/2	0.20	0.54	0.35
20/2	0.20	1.50	0.83
20/2	0.20	3.92	1.17
25/2	0.30	1.03	0.47
25/2	0.30	2.34	0.89
26/2	0.30	1.03	0.52
28/2	0.50	1.78	0.91
28/2	0.50	3.50	1.34
1/3	0.33	1.29	0.52
1/3	0.33	2.06	0.88
1/3	0.33	2.53	1.08
4/3	0.03	0.10	0.02
4/3	0.03	0.14	0.03
4/3	0.03	0.18	0.11
8/3	1.00	2.70	0.98
8/3	1.00	4.50	1.13
20/3	1.00	2.50	1.04
20/3	1.00	5.40	1.15
20/3	1.00	6.50	1.20

Mathematical treatment of the experimental data.

The method of least squares has been employed.[1]

[1] For technique see M. Ezekiel: Methods of Correlation Analysis. New York 1945.

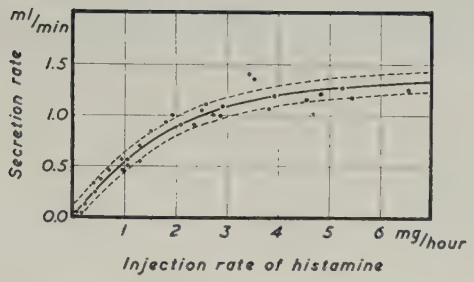


Fig. 14. *The relation between the injection rate of histamine* (r) *and secretion rate of gastric juice* (v). The smoothing curve is that obtained from the eq. $v = 1.37 - 1.38\, e^{-0.5r}$. The dotted lines indicate the "standard error of estimate."

The eq.

$$v = a\,(1 - e^{-kr})$$

may be written in a more general form

$$v = a_0 - a_1 e^{-kr} \tag{21}$$

where the condition that the curve must pass through the origin is abandoned.

Eq. (21) may be written

$$v = a_0 - a_1 u \tag{22}$$

where

$$u = e^{-kr} \tag{23}$$

Eq. (22) gives the following *normal equations*:

$$\begin{cases} n \cdot a_0 - a_1 \Sigma u = \Sigma v & (24) \\ a_0 \Sigma u - a_1 \Sigma u^2 = \Sigma u v & (25) \end{cases}$$

The values of u are calculated from (23) and will depend on the value of k. It is therefore necessary to solve the eq. system (24), (25) for different values of k and calculate the corresponding sum of squares of the differences, d, which will have the following expression:

$$\Sigma d^2 = \Sigma v^2 - a_0 \Sigma v + a_1 \Sigma u v \tag{26}$$

In *Table 2* the dependence of this sum on the value of k indicates that the *least* sum is obtained for a k value of about 0.5. A more exact determination of the value would have no mathematical significance.

Table 2. *The statistical value of eq. (21) for different k-values.*

k-value	Σd^2	a_0	a_1
0.25	0.5670	1.79	1.64
0.50	0.2823	1.37	1.38
0.75	0.2866	1.21	1.35
1.00	0.4093	1.13	1.36

Thus the solution is

$$k = 0.5$$

$$a_0 = 1.37 \pm 0.03$$

and

$$a_1 = 1.38 \pm 0.06$$

Thus

$$v = 1.37 - 1.38 \cdot e^{-0.5\,r} \tag{27}$$

The *index of correlation*, R, is 0.97 ± 0.01.

The *standard error of estimate*, $\sqrt{\frac{\Sigma d^2}{n-2}}$ is 0.09 ml/min. The eq. (27) gives a very good agreement as is seen in *fig. 14*, where the broken lines are the statistical limits given by $\pm$ the standard error of estimate.[1] It is seen from this figure and understood from the a-values, that at an injection rate $r = 0$ the curve has a v-intercept, that is not statistically different from 0, i. e. *the curve passes through its origin.*

Thus it would be correct to write the eq. (27)

$$v = 1.37\,(1 - e^{-0.5\,r}). \tag{28}$$

The experiments above were performed on a single dog. The same results, however, are obtained on other dogs. For example *fig. 15* shows the same relations on this and other dogs and indicates, that *it might be justifiable to consider the above results as having general validity.*

[1] Other mathematical expressions have been correlated with the experimental values but with unsatisfactory results. Examples of such equations are:

$$y = a\,e^{-\frac{1}{x}} + b;$$

$$y = a \cdot \log\,(x + b);$$

$$a\,x^2 + b\,x\,y + c\,y^2 + d\,x + e\,y + f = 0;$$

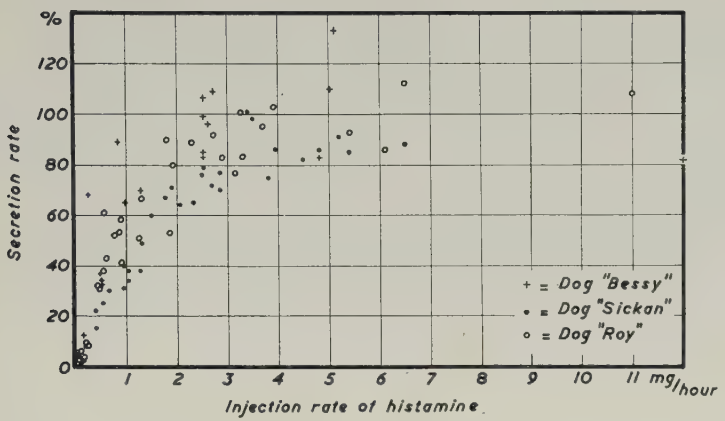


Fig. 15. *A comparison between concentration-action curves from different dogs.*

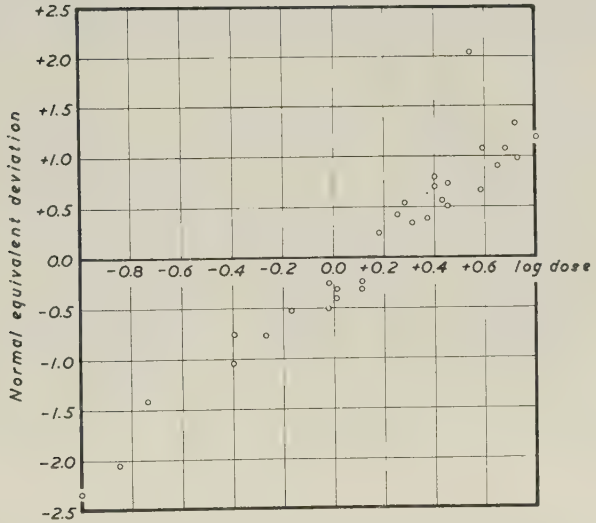


Fig. 16. *The probit transformation of the experimental results in fig. 14.*

These results suggest that *the secretory rate is very closely related to the injection rate* of histamine and to its concentration in the plasma and probably also to the concentration in the mucosa. This is in perfect accordance with the results presented in the previous chapter, where lack of agreement between the stimulus and secretion was only apparent and was due to special experimental circumstances.

Discussion on the concentration-action curve.

Fig. 14 shows the close relation between the injection rate of histamine (i. e. the concentration of histamine) and the secretion rate. This shape of a concentration-action curve is well-known to all physiologists and pharmacologists.

In the discussion of this curve it might be of interest to transform the abscissa (the *dose*) into a logarithmic scale (the *log dose*). With this transformation fig. 14 changes to a slightly *S*-shaped curve. This relation was also reported by Ivy, Hanson & Grossman (1947).

The *S*-curve may be considered as the integral of a normal distribution curve as indicated by the *fig. 16* where the "*normal equivalent deviation*" to the secretion rate[1] is plotted against the log injection rate. This follows a procedure proposed by Gaddum (1933) and would give a straight line if the increments of the secretion rate in relation to the logarithm of the injection rate of histamine resulted in a normal distribution curve. The points in fig. 16 lie practically along a straight line.

This means, in practice, that the procedure presented is a good method for standardizing histamine preparations. The intersection between the straight line and the abscissa in fig. 16 is the corresponding "*LD 50*" — Trevan (1927) — and this dose is a good measure of the activity of the histamine preparation. (Because of the laboriousness of this method it is, however, only of theoretical interest.)

It is of interest to compare the present concentration-action curve with the one obtained when the *pancreas* is stimulated by the continuous intravenous injection of *secretin*. Wilander & Ågren (1932) and Greengard, Stein & Ivy (1941) found an *S*-shaped relation between the secretion rate and the *dose* of secretin. Their technique is similar to the present one, but the resulting curves are different.

It is not possible to explain this disagreement on the basis of our present knowledge.

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[1] "The normal deviation (y) which is equivalent to any given percentage depends on the shape of the normal curve. It is equal to the deviation from the mean, and is measured in standard deviations as units. When the percentage (p) is 50, $y = 0$; when it is greater than 50, y is positive, and when it is less than 50, y is negative. Algebraically its value is given by the equation

$$p = \frac{1}{\sqrt{2\pi}} \int_{-\infty}^{y} e^{-\frac{y^2}{2}} dy$$

(After Gaddum (1933).)

As the volume secreted is produced by the activity of many cells, the mode of reaction of these elements ought to be known before we can disintegrate the resultant action curve.

At the moment we may only give an outline of the possible modes of reaction of the secreting cells:

a) The cells may react uniformly, i. e. they start the secretion at the same dose and have a *graded response* to increasing doses. The concentration-action curve of every individual cell would then be expected to have the same shape as the curve in fig. 14.

b) The cells may have a *quantal*, or all-or-nothing, response, i. e. they start reacting at a certain threshold dose and at once give a maximal response. If the threshold doses were to have a distribution curve skew to the right fig. 14 might be the result.

c) Finally the cells may have a graded response but start secretion at different threshold doses.

It is impossible to say which of these three possibilities is the correct one.

CHAPTER 7.

The Secretion of Gastric Juice after Subcutaneous Injections of Histamine.

As the most usual way of stimulating the gastric glands by histamine is by subcutaneous injection, it seems necessary to compare this route of administration with the continuous intravenous one from a kinetical point of view.

From chapter 4 the time-concentration relation in blood and tissue after a subcutaneous injection was assumed to be known (eqs 8, 9 and fig. 9, page 21). Combining eq. 9 (the tissue concentration) with the relation "tissue concentration"—"secretion rate" (eq. 20, fig. 14, page 33) the kinetics of the secretion rate after a subcutaneous injection of histamine will appear. The mathematical expression of this relationship is, however, rather complicated[1] but the general shape of the curve is much the same as the tissue concentration curve in fig. 9 (page 21).

[1] $v = a\left[1 - e^{-k\,(R_1 e^{m_1 t} + R_2 e^{m_2 t} + H e^{-k_1 t})}\right]$. The meanings of the symbols are the same here as in chapters 4 and 6.

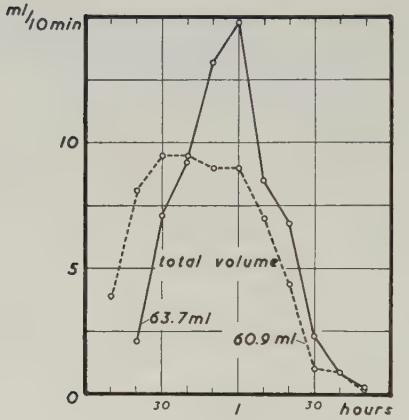


Fig. 17. *Two results from subcutaneous injections of histamine.* (4.0 mg.) The resorption conditions were different, which resulted in different maximal secretion rates. The total amounts secreted were, however, almost equal.

This course is quite well known to everyone who has followed the gastric secretion after a subcutaneous injection of histamine. After a time lag of about 6—10 minutes the secretion rate increases to a maximal value which, in pouch dogs, is obtained after about 25 minutes, when 0.5—1.0 ml of histamine has been injected (the dose is insignificant). In about 60 minutes the period of secretion is over.

The secretion after a single subcutaneous injection of histamine.

Usually, and especially in clinical practice, a single subcutaneous injection of histamine is used. Either the maximal rate of secretion or the total amount secreted is observed.

The maximal rate of secretion.

In chapter 4 it was suggested that the maximal value of the histamine concentration in the tissue was dependent on the resorption rate from the depot (page 23). If this can be kept constant from injection to injection (i. e. k_1 is constant) a close relation between the dose and the maximal rate of secretion would appear. In order to determine such a maximal rate of the secretion it was, however, stated (page 29) that it is necessary to collect at short intervals. Even 10-minutes' intervals may be too long.

If furthermore the resorption varies, the maximal values may differ considerably. In *fig. 17* the same dose (4.0 mg histamine dihydro-

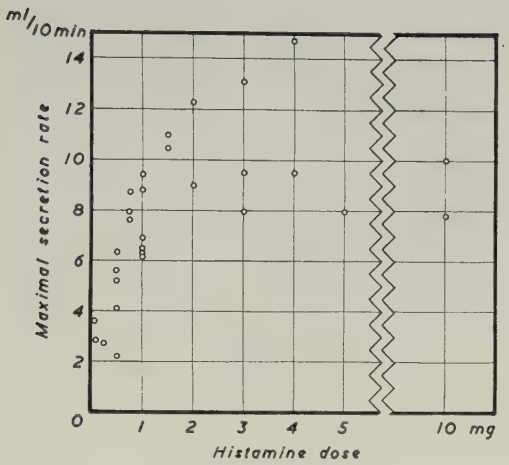


Fig. 18. *The relation between the dose of histamine given subcutaneously and the maximal values of the secretion rate.*

chloride dissolved in 2 ml saline) was given on two different days to the same dog. In the one experiment the injection was made on the thigh where a distinct swelling occurred, in the other one in the inguinal region where the whole volume was spread out. In the second case the maximal secretion was much higher than in the first one, which may depend to some extent on the different conditions of resorption from the two depots. Theoretically we would expect the second curve (the high one) to reach its maximum before the first one (the flat one) but for some unknown reason this was not the case.

If we try, however, to make the resorption conditions as nearly identical as possible, the relation between the dose of histamine and the maximal secretion value (per 10 minutes) will be as good as in *fig. 18.* For small doses this relation seems satisfactory.

The total volume secreted.

The total amount of the drug in the tissue was found to be independent of the resorption rate (independent of k_1) as shown in eq. (16) (page 22): $Z = \frac{k_2}{q} N_0$, where Z = the total amount of the drug present in the tissue after a subcutaneous injection of a dose N_0; k_2 and q are constants.

As, however, the relation between the concentration of histamine in the tissue and the secretion rate is not a direct proportionality

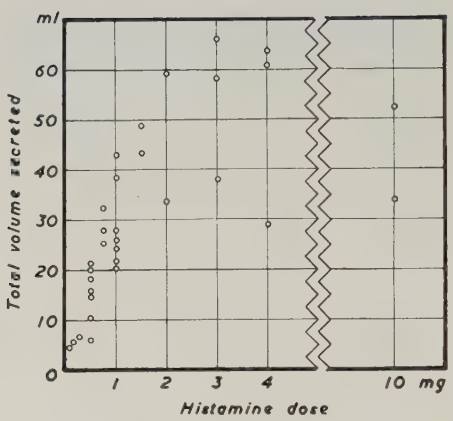


Fig. 19. *The relation between the dose of histamine given subcutaneously and the total volume gastric juice.*

except at the lowest concentrations (eq. 20, page 31 is almost a straight line near to the origin), we might expect to find a close relation between the dose of histamine and the total amount secreted only when the tissue concentrations are kept at a moderate level.

If this is the case the total amount secreted will be proportional to the dose of histamine even though the maximal secretion values per 10 min. period differ. Though the experiments in fig. 17 were performed with rather large doses of histamine (4 mg) they nevertheless show a good agreement in respect to the total volumes (60.9 and 63.7 ml).

It can, however, be concluded that, owing to the shape of the relation "tissue concentration"—"secretion rate," a good agreement between the subcutaneous dose of histamine and the total amount secreted will only be obtained for small doses, which is shown to be the case in *fig. 19.*

"*Continuous histamine test.*"

One interesting consequence arising in this connection is the behaviour of the tissue concentration after *frequently repeated subcutaneous injections.* Such a method of administration was adopted by Bucher, Ivy & Gray (1941) and others, under the name of "*continuous histamine test*". Small doses of histamine were injected subcutaneously every 10 minutes with the intention of obtaining a constant secretion rate. In this case the concentration of histamine in the tissue may be considered as the sum of the concentrations obtained after every individual injection. This is best understood by looking at *fig. 20.* The time lag between an injection of histamine and its maximal concentration in the tissue

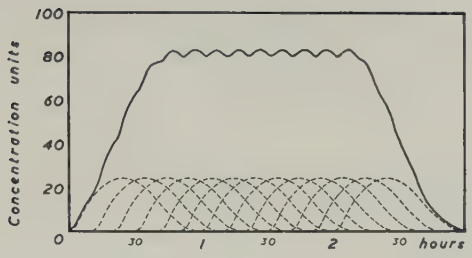


Fig. 20. *The theoretical concentrations of histamine in the tissue after repeated subcutaneous injections.* Every single injection is assumed to give a time-concentration curve like the dotted ones. The sum of these gives the total concentration.

is assumed to be 25 minutes. After about 50—60 minutes the tissue concentration level will oscillate around a mean value. The secretion of gastric juice is supposed to follow these oscillations and will consequently give the same secretion volume for every period of 10 minutes as long as the injections are repeated. This method might therefore be almost as good as the one used in this paper. A disadvantage is the repeated injection injuries.

The method presumes, however, that the resorption rate (the "velocity constant" k_1, page 20) from the injection depot is constant, otherwise the individual dotted curves in fig. 20 will not be identical and a constant secretion rate will not be obtained.

CHAPTER 8.

The Minimal Effective Dose of Histamine Producing Gastric Acid.

It is apparent that histamine acts after its transportation by the blood — Gutowski (1924 b) — to the cells of the mucosa. In a previous chapter the dependence of the secretion rate on the concentration of histamine in blood and mucosa has been demonstrated.

In this connection it seems convenient to discuss a problem which has been a point of controversy for many years, i. e. the minimal effective dose of histamine in the production of gastric acid.

The authors who have given the question any attention have never doubted the existence of a threshold for histamine action on the gastric mucosa but they do not agree as to its level. *Table 3* shows the minimal effective doses as postulated by different authors.

Study of the curve in *fig. 14* (page 33) allows a contribution to the problem to be made.

Table 3. *The minimal effective dose of histamine as reported by different authors.*

Author	Reported threshold dose	Expressed in μg^1/kg/hour
Ivy and Javois (1924)	0.0027 mg[1]/kg/min.	162
Teorell (1933).....	100 γ^2/15 min.[4]	58
Engeström (1935)	$< 5\gamma^3$/kg/15 min.	<12
McElin and Horton (1946)	<0.00003 mg[1]/kg/min.	< 1.8
Ivy, Hanson and Grossman (1947).	0.03 μg^1/kg/min.	1.8
The present author (Dog S).....		3
» » » (Dog R)		0.9
» » » (Dog B)		0.8

[1] Histamine base.
[2] Histamine phosphate.
[3] Histamine dihydrochloride.
[4] On a cat (probably about 2.5 kg in weight).
(Histamine base : histamine phosphate : histamine dihydrochloride = 1 : 2.79 : 1.67.)

If a threshold value exists it will be at the point where this curve cuts the abscissa, i. e. the r value for $v = 0$. The eq. (27) indicates the r value to be 0.01 mg/hour, but the statistical limits of the curve enclose the origin and thus the threshold value is not different from 0. That means, that *the slightest amount of histamine administered by continuous intravenous injection will initiate secretion.*

The least injection rate used in the experiments on the dog shown in fig. 14 was 0.1 mg/hour or 0.005 mg/kg/hour. In another dog injection rates as low as 0.0013 mg/kg/hour/ i. e. 0.0008 mg histamine base/kg/hour were effective. This is the lowest value given in the literature as far as is known to the author (cf. Table 3).

Therefore it seems justifiable to state, that *there is no threshold value for the effect of histamine on gastric secretion.*

It is, however, necessary to point out, that this conclusion is valid only for histamine *administered* to the body. It ought to be kept in mind, that *there exists histamine in the blood of a non-treated dog, which has no secretory effect* as described in chapter 4 (page 24). This histamine is nottaken into account above.

CHAPTER 9.

On the Primary Acidity.

It is well known that the acid produced by the gastric mucosa will be exposed to dilution, neutralization, diffusion and other possible processes, which may alter its original strength. The concentration of the gastric acid as measured in a sample obtained from a whole stomach or a pouch will therefore have a different value than it would have if it were examined at the very moment of secretion. This acid in *statu nascendi* has a concentration, which is usually called the *primary acidity*, (*Pa*). In an analogous way the concentration of the changed acid may be named the *secondary acidity*, (*Sa*).

Ever since HEIDENHAIN and PAVLOV first suggested the existence of a primary acidity two fundamental questions have been raised:

1) what *value* has the Pa and

2) is it *constant* or not, i. e. is it independent of the rate of secretion?

In spite of the many attempts of several authors to answer them the questions are still open.

According to the second question PAVLOV (1898 p. 38—39) and his school thought that the Pa must be constant, a suggestion that he never tried to prove experimentally but in which he had very good reason to believe (cf. chapter 10 page 46). Also cf. HOLLANDER & COWGILL (1931), HOLLANDER (1932, 1934, 1938).

ROSEMAN (1907) was the best known of the opponents of this view. He noticed that in spite of a variability of the Sa the chloride content remained constant, and he considered the secretory process to be an elaboration of a neutral chloride which by some specific stimulation was converted into HCl. The stronger the stimulation the more HCl was formed and the less neutral chloride was left.

His conclusions were also adopted by BERGLUND, JOHNSSON & CHANG (1935) who considered the secretion of the chlorides as the essential part of the parietal secretion.

Experiments performed by the present author, however, suggest (page 71 ff.) that the entrance of chlorides in the gastric juice is influenced not only by the acid secretion but also by diffusion processes.

Other theories about the Pa have also been proposed, but they are only variants of the two mentioned, cf. ENGESTRÖM (1935).

Those workers, who, like Pavlov, believe in a constant strength of the Pa have been interested to know the *value* of it.

According to PAVLOV the decrease of the Pa was due to *dilution and neutralization by mucus*, the effect of which would have a greater significance at low secretion rates. The higher the secretion rate the less the difference between Pa and Sa. Therefore HOLLANDER (1934) tried to evaluate the magnitude of the Pa by *extrapolation* of the Sa values for infinitely large secretion rates. He found that the Pa should be about 170 mN – a most probable value because it is almost *isotonic with blood*. Using the same method GRAY (1943) gives 159 mN as the Pa value.

In a few cases experiments have been made to determine the Pa by a dilution method. The principles were to cause such a high dilution of the primary acid, that the reduction of acidity by dilution with mucus or other admixtures of the gastric juice would be negligible. These observations gave similar results to the extrapolation method, though in a few cases extremely high values were obtained — WILHELMJ, O'BRIEN & HILL (1936), PENNER, HOLLANDER & POST (1940), BANDES, HOLLANDER & GLICKSTEIN (1940).

When TEORELL (1933) proposed his theory of diffusion (the "*diffusion theory*" cf. page 47) another factor, that may reduce the Pa had to be taken into consideration. Using this new principle TEORELL (1940) described another method of determining the Pa. He caught the secreted acid in a *glycocol buffer*, thus causing both a dilution and a prevention of back diffusion of the hydrogen ions. Using this method he concluded that the Pa was constant and almost isotonic with blood (see also the papers by IHRE (1938), ELLIOT, RISHOLM & ÖBRINK (1942) and KAIJSER (1943)), but he also observed much higher values, especially at low secretory rates. These values were, however, too uncertain and not taken into account.

By the method described in chapter 2 it has, however, been possible to make observations at very small secretion rates. Under these new conditions work on the Pa has been carried out by LINDE, TEORELL & ÖBRINK (1947). Their results are of importance for the discussion of the Sa, that follows, and will therefore be recapitulated here.

The primary acidity is probably not constant but becomes higher at low secretory rates. It decreases as the secretion rate increases and approaches a value of about 170 mN asymptotically. *Fig. 21* shows

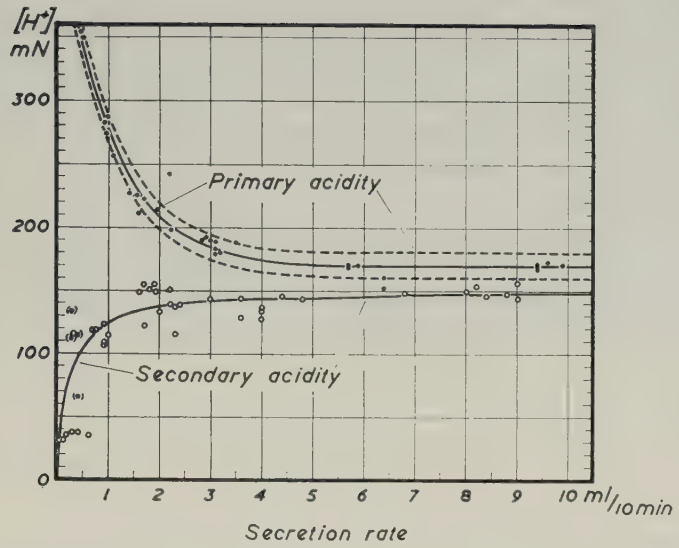


Fig. 21. *The primary acidity in relation to the secretory rate.* The corresponding secondary acidity values from the same dog are plotted as open circles. — After Linde, Teorell & Öbrink (1947).

the relation. In this figure the relation between the secretion rate v and Pa is expressed in

$$Pa = P_\infty + (P_0 - P_\infty)\, e^{-k_1 v} \qquad (29)$$

where P_∞ is the Pa-value for $v = \infty$, P_0 the value for $v = 0$ and k_1 is a constant. The corresponding Sa-values are given for comparison.

The corresponding *chloride values are always somewhat higher* which may depend on the diffusion of *Cl* into the buffer solution.

The significance of the values has been critically discussed with regard to the possibilities of artefacts.

One should note the good agreement between these high Pa-values and some modern theories about the HCl-formation in the mucosa. Bull & Gray (1945) consider the exchange diffusion of the anion from an organic acid for Cl^- to be the essential process in the HCl-formation. The organic acid is formed from glucose and may be pyruvic acid, which must have a concentration of 330 mN in order to be isotonic with blood. Conversion of this into HCl will of course give an HCl-solution of 330 mN which is hypertonic because of the high degree of ionization of hydrochloric acid. In their experiments Linde, Teorell & Öbrink obtained values of about 350 mN.

It should, however, be noticed that the osmotic work required to make such a hypertonic solution, may not have been performed in the buffer solution experiments, as the isotonic volume instilled probably prevented any significant hypertonicity even if all the pyruvic acid was converted to HCl.

As yet therefore, there are no *definite* proofs of the existence of these extremely high Pa values.

As will be seen in chapter 10 it is of no decisive importance for the following whether the Pa increases at low secretory rates or remains constant, and therefore the value of the Pa will mostly be handled as a constant in the following.

CHAPTER 10.

Regulation of the Acidity.

Introduction.

If we assume a *primary acidity* (Pa), the concentration actually observed, the *secondary acidity* (Sa), results from a reduction of the Pa. The difference between Pa and Sa must depend on some mechanism about the nature of which a discussion has been going on for many years.

Pavlov (1898) who was the first to pay any attention to this question, stated that the *mucus* would *neutralize* and *dilute* the secreted acid. He noticed that the acidity decrease was more pronounced at low rates of secretion. The capacity of mucus for binding hydrochloric acid could be detected in a convincing way at the start of an experiment. At the height of the secretion, the mucus was, however, maximally loaded with acid and when the rate decreased at the end of an experiment no decrease in acidity would therefore be possible. This made Pavlov believe in a constant primary acidity (cf. page 43) and in the reduction of acidity by mucus. This theory concerning the mucus seemed very convincing and is still defended. Babkin and his co-workers believe in the neutralizing capacity of mucus.[1]

Boldyreff (1907, 1911, 1914, 1933) has proposed another explanation of the acidity decrease. He observed that under certain conditions

[1] For a good review in this field see the monograph by Babkin (1944).

a *regurgitation of duodenal contents* occurred. This admixture was alkaline and able to neutralize (and of course dilute) the acid. The neutralizing capacity of the duodenal juice is considered to be much higher than that of the mucus. According to IHRE (1938) the content of alkali is about 10 mN in the mucus but 100 mN in the pancreatic juice.

In most cases this explanation of BOLDYREFF is very difficult to exclude. However, experiments have been carried out on man in which precautions have been taken against getting any admixture from the intestine. Cf. IHRE (1938) and ELLIOT, RISHOLM & ÖBRINK (1942).

In the present paper the experiments have been performed on pouch dogs. That means that no account need be taken of any admixture from places outside the pouch.

In 1933 TEORELL put forward his *theory of diffusion*. By experiments on cats' stomachs he came to the conclusion that the gastric mucosa could be considered as similar to a dialysing membrane, through which the hydrogen ions of the juice were exchanged for sodium ions of the blood (or cells) — cf. also TEORELL (1935 a, 1939, 1940, 1947).

His statement was based on the fact that a considerable decrease in the concentration of instilled hydrochloric acid occurred in a tied-off stomach without any appreciable changes in the volume.

Some papers have appeared — IHRE (1938), ELLIOT, RISHOLM & ÖBRINK (1942), KAIJSER (1943), FLENSBORG (1944), SHAY, KOMAROW, SIPLET & FELS (1946) — that give support to the theory of diffusion, but there are unexplained features, a fact which has been pointed out by some authors — see e. g. BABKIN, HEBB & KREUGER (1941), BABKIN (1944). Of great importance are the papers by COPE, COHN & BRENIZER (1943) showing that radioactive sodium is absorbed from a gastric pouch and by COPE, BLATT & BALL (1943) showing that heavy water easily penetrates the gastric mucosa.

This paper is an attempt to throw some light upon remaining problems in the acidity regulation. First, however, it is necessary to give a more detailed account of the theory of diffusion.

The "Diffusion Theory."

First it must be pointed out that diffusion in this case does not necessarily mean a penetration of the hydrogen ions *through* the

mucosal membrane, but that ad- or absorption *may* have the same effect in producing an acidity decrease under special conditions (cf. page 51).

Three ions are of particular interest and they will be considered here. These are the hydrogen, the chloride and the sodium ions. In this chapter only the behaviour of the hydrogen ion will be discussed. According to TEORELL (1947) the theoretical considerations are the following:

As pointed out by GUGGENHEIM it is allowable to consider the diffusion of the different *ions* of HCl and NaCl resp. taking place as undissociated molecules.

Thus the changes in the *concentration* of the hydrogen ions in the stomach are the following:

$$\textit{increment due to secretion} = \frac{v\,C_0}{p + v\,t}\,dt \tag{30}$$

$$\textit{decrement due to diffusion} = -\frac{k\,H}{p + v\,t}\,dt \tag{31}$$

$$\textit{decrement due to dilution} = -\frac{v \cdot dt}{p + v\,t} \cdot H \tag{32}$$

where v is the secretion rate, p is the volume of non-acid material present in the stomach when the experiment starts (test meal volume), C_0 the strength of the secreted acid (the primary acidity at the secretion rate v), H the actual hydrogen ion concentration, t the time and k a permeability coefficient including the diffusion coefficient, the diffusion area and the thickness (resistance) of the membrane. The total changes of the hydrogen ion concentration will be

$$\frac{dH}{dt} = \frac{v\,C_0 - (k + v)\,H}{p + v\,t} \tag{33}$$

The solution of this differential eq. will be

$$H = \frac{C_0}{\frac{k}{v} + 1}\left[1 - \frac{1}{\left(1 + \frac{v\,t}{p}\right)^{1+\frac{k}{v}}}\right] \tag{34}$$

If no test meal volumes are present

$$H_{p=0} = \frac{C_0}{\frac{k}{v} + 1} \tag{35}$$

This means that the secondary acidity is not dependant on the time, but only on the secretory rate.

By putting $v = 0$ in eq. (35), $H = 0$, and when $v = \infty$, $H = C_0$.

That means, that the diffusion (like the neutralization and dilution) is of greater significance at low secretory rates.

The deduction made above, which is to be found in the recent paper by TEORELL (1947) assumes the secreted volume to be instantly and thoroughly mixed with the volumes previously secreted. This makes it justifiable to put the "*decrement due to diffusion*" in eq. (31) as proportional to H.

If on the contrary no mixing occurs the diffusion gradient will always be $C_0 - 0$[1] (instead of $H - 0$)[1] as it will only be the freshly secreted volume that is in contact with the diffusion area.

This proposition makes the eq. (33) change to

$$\frac{dH}{dt} = \frac{(v - k)\,C_0 - v\,H}{p + v\,t} \tag{36}$$

which gives

$$H = C_0\left(1 - \frac{k}{v}\right)\left[1 - \frac{1}{1 + \frac{v\,t}{p}}\right] \tag{37}$$

or if $p = 0$

$$H = C_0\left(1 - \frac{k}{v}\right) \tag{38}$$

Here $H = 0$ when $v = k$ and $H = C_0$ when $v = \infty$.

Eq. (38) represents a hyperbolic curve approaching $H = C_0$ for increasing v values and cutting the v-axis at $v = k$ (cf. page 69).

A curve, almost identical with this one is given by GRAY, BUCHER & HARMAN (1941) but on a different theoretical basis (cf. page 69).

The value of the primary acidity (C_0).

An important question is the value of C_0. In the previous chapter it was found that C_0 varied with the secretion rate in accordance with the eq. (29):

$$Pa = P_\infty + (P_0 - P_\infty)\,e^{-k_1 v}$$

where Pa is the primary acidity, P_∞ the Pa value for $v = \infty$, P_0 the Pa value for $v = 0$.

[1] The hydrogen ion concentration in blood is very low in comparison to that of the gastric acid and can be neglected ($= 0$).

For the solution of the differential eqs. (33) and (36) it is of no importance whether C_0 is a constant or not. The main thing is that it is constant for one single v value. Therefore we may substitute Pa for C_0. Eq. (35) will then be

$$H = \frac{P_\infty + (P_0 - P_\infty)\, e^{-k_1 v}}{\frac{k}{v} + 1} \tag{39}$$

The curve corresponding to this equation is roughly similar in shape to that given by (35), as for $v = 0$, $H = 0$, and for $v = \infty$, $H = P_\infty$.

Furthermore the derivates *at the origin* ($v = 0$) are:

for (35)

$$\frac{dH}{dv} = \frac{C_0}{k} \tag{40}$$

and

$$\frac{d^2H}{dv^2} = -\frac{2\,C_0}{k^2} \tag{41}$$

and for (39)

$$\frac{dH}{dv} = \frac{P_0}{k} \tag{42}$$

and

$$\frac{d^2H}{dv^2} = -\frac{2\,P_0}{k^2} \tag{43}$$

With the constants involved, the general shapes of the two curves will be very similar. As, however, P_0 is greater than C_0 the curve for the *Sa* will rise more quickly (cf. eqs. (40) and (42)).

In conclusion it may be said that it does not matter whether the *Pa* is constant or not, as the general shape of the curve relating the *Sa* to the secretion rate will be almost the same in both cases.

Experimental data.

In *fig. 22* the relation between the secretion rate and the secondary acidity is given. Every point in this curve is obtained from several determinations of a steady state level of the secretion rate and the corresponding acidity value (cf. fig. 12, page 27). (Similar curves from other dogs are found in fig. 25, page 60). The significance of these results will now be discussed.

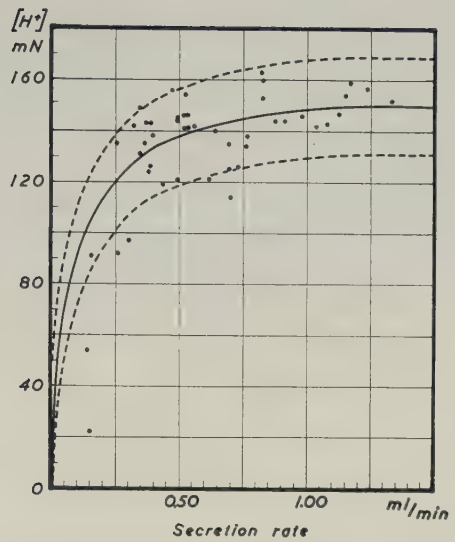


Fig. 22. *Relation between the secondary acidity and the secretion rate* (dog S). The full line is the curve derived from the diffusion theory which best fits the actual observations. The dotted lines denote the "standard error of estimate."

In the foregoing pages the factors that may reduce the strength of the *Pa* have been mentioned, they are:

1) *Dilution*,
2) *Neutralization* and
3) *Diffusion*.

No other possibilities appear to exist. Every factor mentioned in the literature can be included in one or more of these three categories. It should be pointed out, that diffusion is used in such a wide sense, that adsorption under special conditions may be described as diffusion. The reason is, that in the technique used (see chapter 2) an adsorption that will load the mucosa or its mucus lining gives rise to no problem as the results discussed are not observed until a convenient time has elapsed and the secretion rate and acidity have become constant. That means that the mucosa is already loaded and can no longer cause a further adsorption. If, however, the adsorbed amount should be given off on the other side (the blood side or nutrition side) more acid could be adsorbed from the gastric contents. The remaining process will then be analogous to that of diffusion.

If on the other hand a continuous secretion of mucus should occur thus adsorbing acid during the entire experiment, this mucus secreted

would be obtained in the measuring cylinder (a continuous accumulation of mucus within the pouch would be impossible). In the present experiments the acidity values, which will be discussed, are determined as the total acidity (titration with brom-thymol-blue pH range 6.0—7.6 cf. chapter 3, page 15) and therefore any acid which may have been adsorbed or neutralized by mucus has also been detected. Only a very small amount can still be fixed to mucus according to the acid-base binding curves for mucus made by Ihre (1938).

Thus there remain only the three possibilities mentioned above: dilution, neutralization and diffusion.

1. *Dilution.*

Every non-acid admixture to the stomach contents acts as a diluent. Examples may be found in test meals or washing fluids. In the present experiments no test meal is used, but the pouch is washed out with saline before every experiment. During the first minutes of an experiment this will of course dilute the secreted acid, but the effect will be more and more insignificant as the saline is forced out, and when the acidity has become constant we have reason to believe that it has no more influence.

Those components of the gastric secretion which are non-acid may also act as diluents. Such constituents are *mucus*, *enzymes* and "*diluting secretion*" — "Verdünnungssekretion" — (if such a secretion does exist).

As the main question is whether there is something secreted which may dilute the parietal secretion, it is of no importance to know if this is mucus, enzymes or some other "Verdünnungssekretion." Therefore we call all these *diluting secretions.*

The diluting secretions.

Very few authors have tried to prove the existence of a diluting secretion after histamine stimulation — cf. Hollander & Cowgill (1931), Engeström (1935) and Hollander (1938).

Experiments concerning the primary acidity by Linde, Teorell & Öbrink (1947) seem, however, to disprove the existence of any important diluting secretion.

By carefully maintaining constant secretion rates they were able to prove that the difference between the primary and the secondary acidity was not due to any diluting admixture to the primary acid.

Only at extremely low secretory rates, less than 0.01 ml/min, a slight diluting secretion was significant. The order of such a secretion would be about 0.01 ml/min, from which it follows that dilution would be significant only for secretion rates of the order of 0.01—0.05 ml/min (vide infra).

It might be stated that in the present experiments *any significant diluting secretion can be excluded* with the exception of this unimportant amount of non-acid admixture.

2. *Neutralization.*

The only constituents of the gastric juice that could have any neutralizing effects would have to contain some basic radicals. Such are present in the proteins and in the mucus owing to their ampholytic character. The acid-binding capacity of mucus is very slight according to Ihre (1938). The concentration of bicarbonate in the mucus has been determined with the following results: Bolton & Goodhart (1931) — 39 mN; Webster (1933) — 16.8 mN; Baxter (1934) — 11.2—14.0 mN, Ihre (1938) — 3.2—10.8 mN and Mahlo (1938), who considers the mucus to have an acid reaction.

The bicarbonate reacts with the hydrochloric acid to form sodium chloride and carbonic acid, which in a second step would give water and carbon dioxide. This gas would easily be re-absorbed or given off into the air and thus escape a determination.

In this connection it should be pointed out that such a neutralizing effect may also have occurred in the estimation of the primary acidity — Linde, Teorell & Öbrink (1947) — and is therefore included in the calculated value of the *Pa*.

If, in spite of the above, the mucus should lower the acidity could it be responsible for the acidity decrease in fig. 22? A secretion rate of 0.06 ml/min corresponds to an acidity value of 38 mN. That means a decrease of 132 mN from a constant C_0-value of 170 mN. If the mucus were responsible for this and is considered to contain 10 mN of alkali, its volume-rate (m ml/min) can be calculated in the following way:

$$(0.06 - m) \times 170 - m \times 10 = 0.06 \times 38$$

or

$$m = 0.04 \text{ ml/min.}$$

At such a secretion rate it would also act as a diluting fluid, which

has been disproved above (page 52). If the secretion of mucus should take place within probable limits (0.01 ml/min) the alkaline strength of it (n mN) would be

$$0.05 \times 170 - 0.01 \times n = 0.06 \times 38$$

or

$$n = 622 \text{ mN},$$

which is in absolute disagreement with earlier investigations.

Thus, it may be stated that *the effect of the mucus in regulating acidity can generally be neglected.*

This view is contrary to that of many investigators. BABKIN and his collaborators have made many experiments showing the influence of the mucus. Here it would be of interest to discuss one paper of GRANT (1942) which is also referred to by BABKIN (1944 page 406).

GRANT introduced hydrochloric acid into a cat's stomach and observed the acidity decrease, but in order to prove that the decrease is dependant upon the neutralizing power of the mucus she collected mucus after stimulation with 1 % acetic acid or 70 % alcohol. This mucus was exposed to 130 mN HCl *in vitro* for 15 minutes. The acidity decrease was determined as well as the calcium uptake from the mucus, which always contains Ca (GRANT 1941). After this period a fresh volume of 130 mN HCl is mixed with the same mucus and the procedure is repeated three times.

The result is an acidity reduction which is most significant in the first, less in the second and least in the third period. The calcium uptake shows the same relations. *Table 4* gives the data.

Even so the neutralizing power of the mucus can be doubted. The calcium uptake from the mucus may be a matter of diffusion. The hydrogen ions could also diffuse into the mucus.

As we know from Table 4 to what degree Ca^{++} has diffused out from the mucus we can calculate the amount of H^{+}-ions, which have entered the mucus in the same time. The calculated acidity reduction is given in column 8 Table 4 and shows that the total decrease *is almost entirely a matter of diffusion.* Only the small differences between the observed and the calculated values may be a result of the bicarbonate content of the mucus. This is a quite different explanation than that offered by GRANT.

Table 4. *The neutralizing capacity of mucus.* (*Columns 1—7 from* GRANT (*1942*), *columns 8—9 are calculated by the author.*)

Cat No.	Period of 15 min	Volume of 130 mN HCl in contact with the mucus ml	Volume of the mucus ml	Calcium in mucus mg/100 ml	Calcium uptake mg/100 ml	Acidity reduction observed mN	Acidity reduction calculated mN	Difference mN
2	1	7.0	3.0	6.00	0.90	40	33	+ 7
	2				0.65	15	14	+ 1
	3				0.45	6	6	0
3	1	7.2	3.6	10.10	1.85	46	38	+ 8
	2				1.15	24	14	+10
	3				0.70	6	7	− 1
4	1	7.0	5.2	10.48	1.60	56	38	+18
	2				1.30	29	24	+ 5
	3				0.80	11	12	− 1
5	1	7.0	3.3	8.75	1.40	37	36	+ 1
	2				1.15	20	16	+ 4
	3				0.80	8	6	+ 2
6	1	7.0	4.5	7.37	1.65	53	46	+ 7
	2				1.30	28	22	+ 6
	3				0.60	14	8	+ 6

The secretion rate of mucus.

It would be of great interest to know how much mucus is secreted during an experiment. This demands a method of determining the mucus, but specific methods do not exist at present. Many suggestions have been made, and in the present paper four methods have been used.

A) Titration curves: The main question is to what extent the mucus can neutralize the acid. This might be detected by making an ordinary titration curve of the acid-binding capacity.

Many such curves were made on samples of gastric juice from the pouch dogs, but none showed any properties that could originate from the mucus. Every curve was perfectly flat.

B) Calcium analyses: GRANT (1941, 1942, 1944) states that the content of calcium should be directly proportional to the acidity decrease and to the amount of mucus, and therefore Ca has been

Table 5. ***Calcium in the histamine induced gastric secretion.***

Secretion rate ml/min	Time after starting the experiment hours	Calcium concentration mg/100 ml
0.10	0^{50}	1.66
	1^{20}	0.25
	1^{50}	0.00
	2^{10}	0.00
	2^{30}	0.00
	2^{50}	0.00
0.81	0^{20}	0.77
	0^{50}	0.38
	1^{10}	0.00
	1^{20}	0.00
	2^{00}	0.00

determined. The results from one dog are found in *Table 5*. There it may be seen that in the first few periods of 10 minutes some Ca is detectable but it then disappears altogether from the gastric juice. *The mucus (as Ca) seems to have been washed out.*

C) Nitrogen analyses: Mucus is a protein-like substance and will thus be detectable by nitrogen analysis. These values also decrease during an experiment as if a washing-out effect existed. Yet, nitrogen remains present during the whole experiment, and some part of it may originate from a protein. If *all* the nitrogen is considered to originate from proteins a rough estimate of the acid-binding capacity would be obtained by a titration curve of another protein mixture of a known strength.

A suspension of hen's egg-white containing 1 mg of nitrogen per ml has been used. The result is given in *fig. 23*, which shows the base-acid-binding capacity of 5 ml of the protein solution expressed in ml of a tenth normal (N/10) of a proteolyte. Comparing this with *Table 6* and *fig. 24* where the nitrogen content of the gastric secretions are plotted against the secretion rate, it is easy to understand the relatively low acid-binding capacity of the juice.

Consequently, *this mode of determination also indicates the very slight effect of the mucus upon the hydrochloric acid.*

D) Reduction power analyses: The mucus is considered to be a mucoprotein and will consequently contain a hexose group. It will therefore show a *reduction power* after hydrolysis.

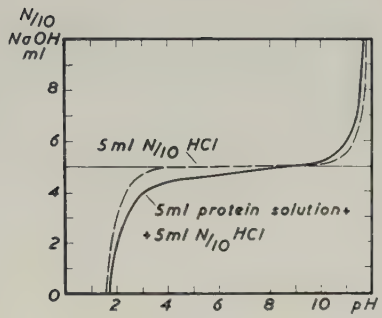


Fig. 23. *The acid-base-binding capacity of a protein solution* (hens egg-white) *containing 1 mg nitrogen per ml.*

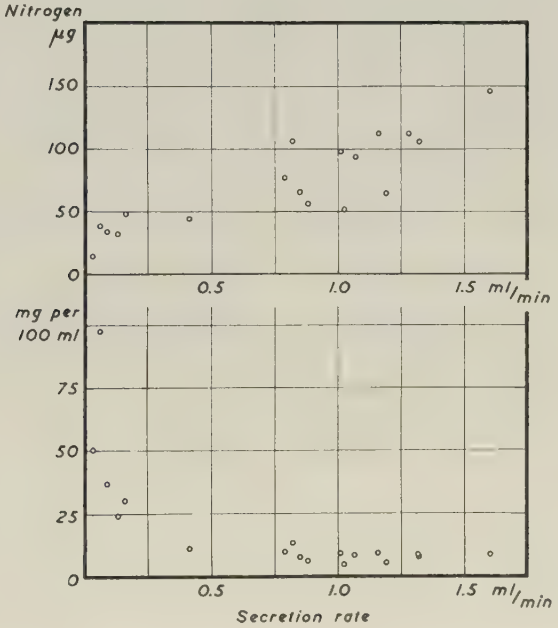


Fig. 24. *The concentration and total amount of nitrogen in relation to the secretion rate.*

The results are expressed in mg glucose per 100 ml juice and are seen in *Table 7*. Ihre (1938) who investigated almost pure gastric mucus found 191 mg/100 ml. That means that the present mucus is diluted $>$ 1: 20. The volume of this mucus would then be in the order of 0.01 ml.

Table 6. *The pepsin content and N-concentration in the gastric juice (cf. fig. 24).*

Secretion rate ml/min	Pepsin mg protein/ml/min	Nitrogen mg/100 ml
0.06	—	98.0
0.16	1.23	30.0
0.41	—	10.9
0.79	—	9.8
0.82	1.45	13.1
0.85	0.64	7.7
0.88	0.47	6.2
1.01	0.89	9.7
1.02	0.73	5.0
1.07	0.98	8.8
1.16	0.83	9.8
1.19	0.57	5.4
1.28	0.78	8.8
1.32	0.51	8.0
1.60	0.77	9.1

Table 7. *The reducing power (glucose) of histamine induced gastric juice.*

Secretion rate ml/min	Reducing power mg glucose/100 ml
0.24	8.3
0.33	6.3
0.62	7.6

It is the opinion of the author, that all these experiments together give support to the view, that *the acid-binding capacity of the mucus after stimulation with histamine is of very little importance.*

The pepsin content of the histamine induced gastric juice.

It might be of interest to know the amount of pepsin secreted by the present stimulation method, as a large concentration might be responsible for an acidity decrease.

From the titration curves related above, we cannot expect to find any considerable amounts as the pepsin ought to show up in these experimental curves as well as the mucus.

Unlike the mucus, pepsin is, however, rather easy to determine and the amount present has been analyzed when the secretion rates have been held constant. It is expressed in mg protein that has been digested by 1 ml gastric juice during 1 minute (cf. Methods, page 16).

The results are collected in Table 6, where we also see the nitrogen content of the same samples.

As expected, the values are rather small and seem to be independent of the secretory rate and the stimulation. This is in accordance with Björkman, Nordén & Uvnäs (1943) who consider that histamine does not stimulate or inhibit pepsin secretion.

In a few cases the peptic power runs parallel to the nitrogen concentration. As mentioned above this non-parietal secretion can only be of very slight importance in the acidity regulation.

3. *Diffusion.*

The third factor responsible for the acidity decrease will now be discussed. The first two have been considered to be of negligible importance and thus the diffusion may be the main factor. How do the experiments agree with the theoretical considerations from the diffusion theory?

One of the best tests for such a question is to calculate the correlation between the theoretical equation and the experimental data.

Eq. (35)

$$H = \frac{C_0}{\frac{k}{v} + 1}$$

may be transformed to

$$v C_0 - H k = H v. \tag{44}$$

By using the methods of least squares we obtain the following normal equations:

$$\begin{cases} C_0 \Sigma v^2 - k \Sigma H v = \Sigma H v^2 & (45) \\ C_0 \Sigma H v - k \Sigma H^2 = \Sigma H^2 v. & (46) \end{cases}$$

Solving this is a matter of routine and will be omitted here. The results for three dogs are collected in *Table 8* and *Fig. 22* and *25*.

In these figures eq. (35) is drawn as full lines and the limits within $\pm$ *the standard error of estimate* as broken ones. The agreement be-

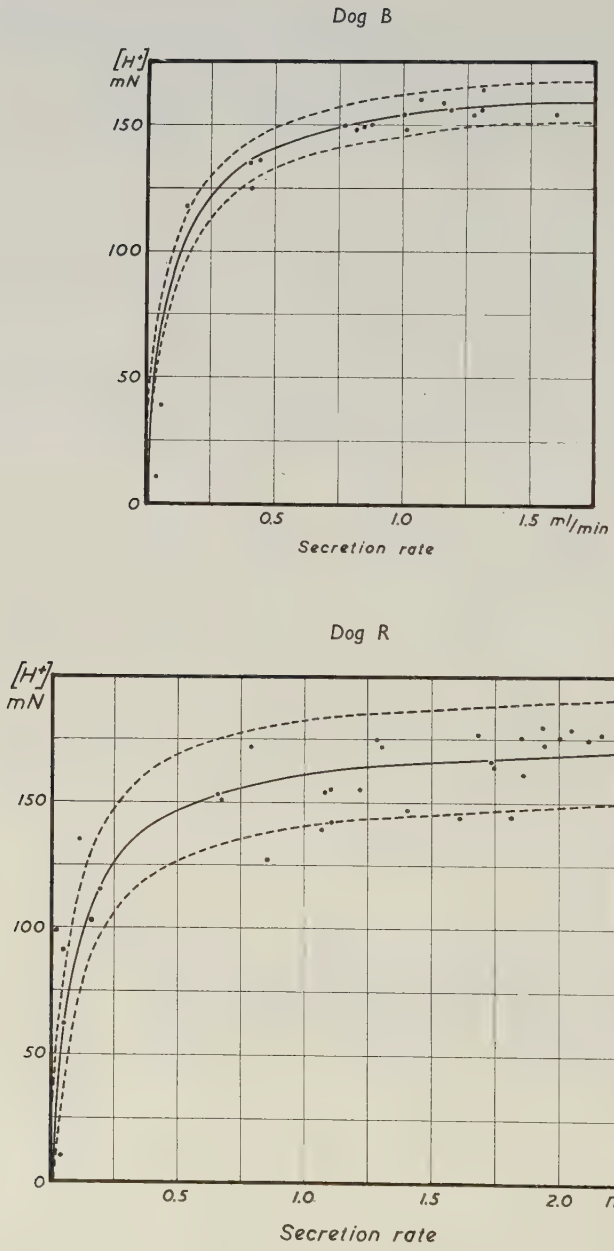


Fig. 25. *Relation between the secondary acidity and the secretion rate.*

tween the theoretical function and the experimental data is good. Only at the lowest secretion rates is the actual acidity somewhat lower than the calculated one. This may partly be due to experimental errors but here the small amount of diluting fluid, which can never be excluded, and which has been discussed above, may have had some influence.

Table 8. *Statistical data to fig. 22 and 25.*

$$\textit{Regression line: } H = \frac{C_0}{\frac{k}{v} + 1}$$

(*H = secondary acidity*, mN; *C_0 = primary acidity (a constant)*, *k = permeability coefficient (a constant) and v = secretion rate, ml/min*)

	Dog S (n = 47)	Dog R (n = 33)	Dog B (n = 19)
C_0 (mN)	160±5	177±6	168±1
k (ml/min)	0.08±0.02	0.10±0.06	0.10±0.02
Standard error of estimate	±19	±21	±8
Index of correlation	0.70±0.08	0.83±0.06	0.98±0.01

(n = number of observations)

We ought, however, to suspect that in the experiments with these low secretory rates the "washing out" of mucus and enzymes, which have collected during the hours preceeding the experiment has been ineffective. Therefore, in a few cases, the pouch has been washed out with 170 mN HCl (instead of saline) before starting the histamine injection. This different procedure gave, however, the same result indicating that all the washing fluid was poured out and no acid-binding capacity was left in the pouch to cause the low acidity values. This must therefore be a result of a very slight dilution superimposed on the main factor — diffusion.

Influence of time.

There are some special investigations, which ought to be considered. From the eq. (35) it is suggested that time is of no significance in the acidity reduction, i. e. *the mode of sampling is of no importance.* The present experiments are usually carried out by letting the juice flow out of the pouch continuously, and therefore controls have been made

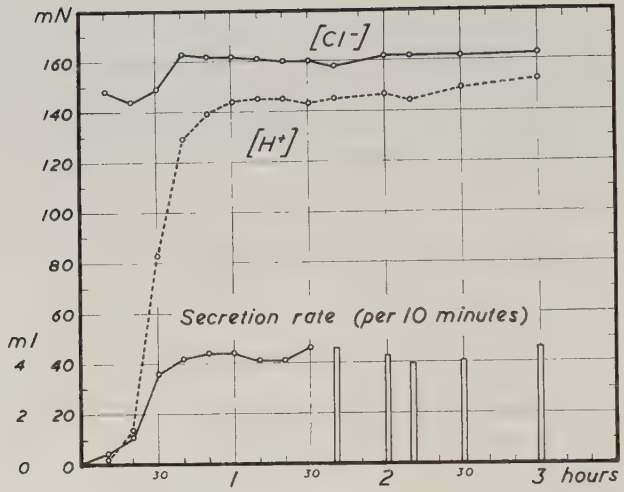


Fig. 26. *Experiment showing the insignificance of mode of sampling on the acidity and the chloride.* Between 0^{00}—1^{30} hrs there was a continuous outflow from the pouch. After 1^{30} the collection was intermittent (10, 20, 10, 20, 30 minutes).

in which the juice was recorded in an intermittent way. *Fig. 26* shows the results. The acidity values were the same after a continuous and an intermittent collection. Any occlusion of the pouch was of no importance, i. e. *the time is insignificant for the acidity reduction* — cf. Gudiksen (1943), Teorell (1947). This invalidates the objections by Welin & Frisk (1936) and Babkin, Hebb & Kreuger (1941) that normally there cannot be sufficient time for diffusion to occur.

Apparent inconsistencies of the diffusion theory.

In some papers some inconsistencies have been remarked, that tend to invalidate the hypothesis of diffusion. One of these is the observation made by Pavlov, that the acidity at the beginning of an experiment was very low and increased when the secretion rate increased. At the end of an experiment, however, the decrease in the secretion rate was not followed by a similar decrease in the acidity. In the present experiments the same thing has occurred. How can that possibly fit in with the diffusion theory? In fact, these circumstances have been one of the things which have been most difficult to explain.

The experiments with steady state values, however, made it clear that the relation between secretion rate and acidity is very close.

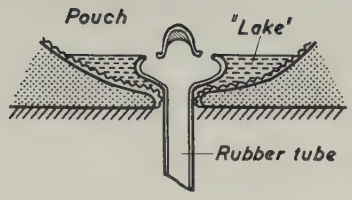


Fig. 27. *The position of the Pezzer catheter in the pouch.*

There must be some explanation for the inconsistency mentioned and it might be the following:

The "lake"-hypothesis. Every attempt to empty *completely* a stomach or a pouch is unsuccessful. There will always be some volume left. This is true of present experiments too. From *fig. 27* the position of the Pezzer catheter in the pouch is seen. In addition to the volume resting in crypts and elsewhere on the mucosa a kind of a "*lake*" is formed around the catheter (see the fig.). The freshly secreted juice runs into this "lake" and some part of the content of the "lake" is drained through the tube.

Experiments have been carried out to determine the size of this "lake" with phenol red (for the method see p. 18), and for one of the dogs the average volume determination was 4.0 ml. This very dog showed the typical curves of the acidity described by Pavlov as related above. One of them is seen in *fig. 28* (curve 1).

The "lake" causes a *time lag* in the sampling of the juice. If we know the secretion rate and the size of the "lake" and if the secreted volume drives the "lake" out entirely before it enters the tube, and if *no mixing* of the secreted volume and the "lake content" takes place, we are able to calculate this time lag.

When the secretion rate is constant and everything else is also constant this time lag is of no importance. This can be shown by putting $t = \infty$ in eq. (34). It does not matter whether two observed values are coincident or not as no change occurs during the time. In the case of a varying secretion rate, however, as in the beginning and at the end of an experiment it will be most necessary to take the time lag into account.

In fig. 28 the typical curve shows that the acidity does not decrease at the end of the experiment (curve 1), as might be expected from the diffusion theory (curve 2). If, however, the time lag is considered, a curve more like the actual one is obtained (curve 3).

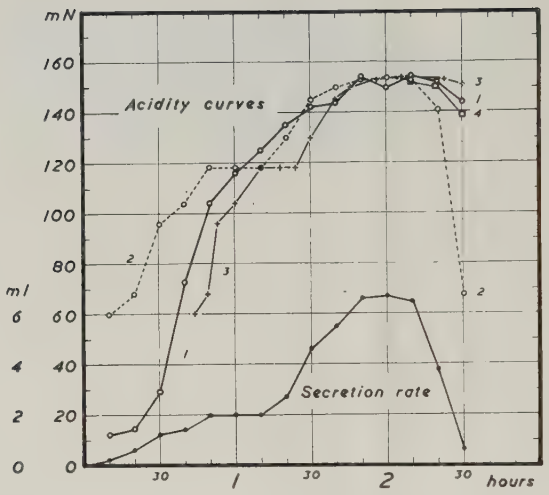


Fig. 28. *A typical experiment with a continuous secretion.* (Two secretion rates were established.) The acidity curve (1) did not decrease at the end of the experiment as expected from the diffusion theory (2). If this curve (2) is corrected for the effects of the "lake" (see fig. 27) one obtains the curves (3) or (4). (See text.)

If in addition to the above a *complete mixing* occurs between the content in the "lake" and the secreted amount, a somewhat different result will occur. This offers a much more complicated problem but may be solved in the following way:

Consider the secretion rate to be constant under a 2-minutes period (v ml/min): The secreted acid has a secondary acidity of H_1 mN. The "lake" contains p ml and at the beginning of the period its concentration is H_0 mN.

I) First consider the concentration changes in the "lake":

$$\textit{increment due to secretion} = \frac{v\,H_1}{p}\,dt \tag{47}$$

$$\textit{decrement due to dilution} = -\frac{v\,dt}{p}\cdot H \tag{48}$$

This makes the concentration increase

$$dH = \frac{v\,(H_1 - H)}{p}\cdot dt \tag{49}$$

The solution gives

$$H = H_1 - (H_1 - H_0)\,e^{-\frac{vt}{p}} \tag{50}$$

II) The amount of hydrochloric acid (M m.eq.) driven through the catheter is calculated from

$$\int_{t=0}^{t=2} d\,M = \int_{t=0}^{t=2} v\,H\,dt \tag{51}$$

which gives

$$M = 2\,v\,H_1 - p\,(H_1 - H_0)\,(1 - e^{-\frac{2\,v}{p}}). \tag{52}$$

When the amount has been calculated according to this formula we start with a new 2-minute period with a new v-value but with H_0 substituted by the H-value in (50) which corresponded to $t = 2$ min. So the calculations may continue for five periods. If the total amount for these ten minutes is divided by the total volume we get the concentration in the 10 minute sample. This procedure led to the construction of the curve 4 in fig. 28. This curve has been calculated only for the descending part of the experiment.

In reality the postulate of "no mixing" and "complete mixing" in the "lake" are extremities, the truth lying somewhere in between.

This "lake-theory" was supported by the following experiments: At the end of an experiment when the last sample was obtained, a small pipette was introduced into the pouch and brought in contact with the mucosa in the upper part of the pouch. A small volume of juice which collected in the pipette showed an acidity value which was considerably lower than that of the last sample obtained from the "lake" contents. Results like the following were obtained (the first number is the last "lake" acidity value, the one in brackets is the corresponding "pipette-value"): 139 (105) mN, 135 (25) mN, 116 (58) mN.

We have now seen that the apparent inconsistency in the acidity curve can be explained by the existence of a "diluting" volume, which we have called the "lake".

That this "lake" is insignificant in the steady state values of the acidity even at very low secretory rates was shown in the experiment related above, where a washing with 170 mN HCl instead of NaCl gave the same constant acidity value.

Thus the continuous intravenous technique has undoubtedly shown that *a definite relation exists between the secretion rate and the secondary acidity.*

A conclusion, that can be drawn from the above is that *the diffusion process takes place at the very spot* where the acid was secreted. *The time required for the juice to run along the mucosal surface does not influence the resulting acidity* (cf. page 61).

Such a consideration is supported by an experiment, in which the dog was laid on its back during the experiment. In the erect position one part of the surface where the juice collects is formed by the head of the rubber tube (cf. fig. 27), where no diffusion can take place. When the dog is in a recumbent position, however, the "lake" will be in contact only with a mucous membrane. In spite of this the acidity was uninfluenced by the arrangement. The result was expected and in accordance with the diffusion theory.

Resorption of hydrogen ions in the non-secreting pouch.

In a whole stomach pouch of a cat TEORELL (1933) found an acidity decrease of an instilled isotonic solution of HCl (170 mN). He ascribed this phenomenon to a diffusion of the hydrogen ions through the gastric mucosa. IHRE (1938), ELLIOT, RISHOLM & ÖBRINK (1942) confirmed his results on adults and KAIJSER (1943) on children. In stomach pouches of dogs BOLDYREFF (1934) failed to get any decrease in acidity of an instilled acid. He, however, used very large volumes (75 ml) which made the influence of the diffusion mechanism insignificant. This was also pointed out by TEORELL (1935 a) (vide infra).

The present author has made the same instillation experiments as Boldyreff but with much smaller volumes (10, 15 and 20 ml). In *fig. 29* a typical experiment on *dog R* is seen. The acidity decrease is almost exponential (cf. fig. 29 a) and could be expressed

$$H = H_0 e^{-\frac{c\,t}{p}} \tag{53}$$

where H = the acidity in the pouch, H_0 = the initial concentration of the instilled acid, t = time (in minutes), c = the permeability coefficient (ml min^{-1}) and p = volume (ml).

The value of $\frac{c}{p}$ can be determined from the slope of the curve in fig. 29 b. It is $\frac{c}{p} = 0.013\ min^{-1}$.

By multiplying this by the average volume in the experiment (8 ml) one gets the *permeability coefficient* $c = 8 \times 0.013 = 0.10$ ml min^{-1}.

This may be compared with the permeability coefficient in *eq.* (35), and *Table 8 dog R*, which is also 0.10 ml min^{-1}.

These findings are further *support for the theory of diffusion as a main factor in the acidity regulation.*

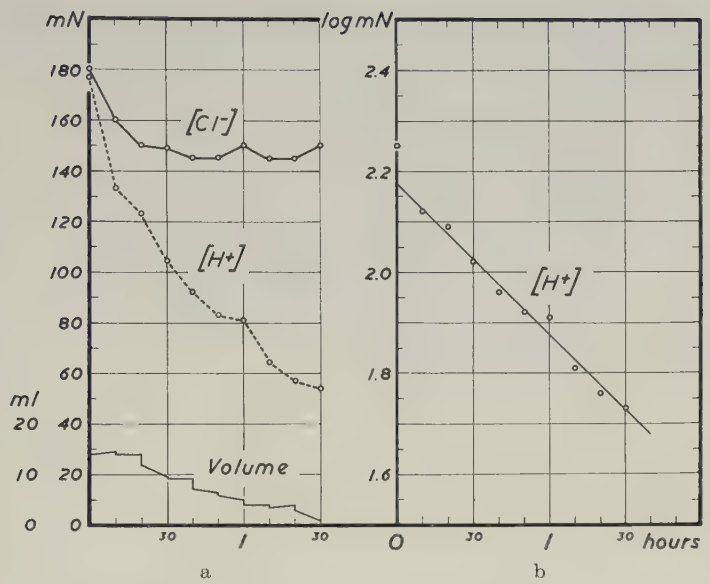


Fig. 29. *An instillation experiment in a cat's stomach of 170 mN HCl.* a) The experimental results. b) The logarithm of the acidity values. The slope of this free hand line is expressed by the "permeability coefficient" (cf. eq. 53).

According to the formula (53) one can easily calculate what would happen in the above mentioned dog R if 75 ml of 170 mN HCl were introduced in the pouch as in Boldyreff's experiments. (In fact such a large volume would cause a severe distension of the pouch.)

After 60 minutes we should obtain

$$H = 170 \cdot e^{-\frac{0.10 \cdot 60}{75}} = 169 \text{ mN},$$

i. e. the acidity decrease would scarcely be detectable and consequently the findings of Boldyreff do not contradict the diffusion theory.

CHAPTER 11.

Total Output of Hydrochloric Acid in Relation to the Secretion Rate.

Theoretical considerations.

A computation of the total amount of HCl obtained from a gastric pouch after histamine stimulation leads to some interesting conclusions, which will be described here.

When the secretion is going on at a *constant rate* the eq. (35) is valid

$$H = \frac{C_0}{\frac{k}{v} + 1}$$

The total amount will therefore be

$$v\,H = \frac{v \cdot C_0}{\frac{k}{v} + 1} = vC_0 \left(\frac{1}{1 + \frac{k}{v}} \right) \tag{54}$$

which could be transformed to

$$v\,H = v\,C_0 - k \left(\frac{C_0}{\frac{k}{v} + 1} \right) \tag{55}$$

or by substituting (35)

$$v\,H = v\,C_0 - k\,H \tag{56}$$

The expression $v\,C_0$ is the HCl secreted per minute and $k\,H$ is the amount of hydrogen ions diffusing out of the stomach. The difference $(v\,C_0 - k\,H)$ is the HCl actually obtained. Graphical representations of these functions, "*HCl secreted*", "H^+ *diffused*" and "*HCl obtained*" are given in *fig. 30.*

Putting $\frac{k}{v} \ll 1$ in eq. (54) one can approximate

$$\frac{1}{1 + \frac{k}{v}} = 1 - \frac{k}{v} \tag{57}$$

A transformation of (54) will therefore give

$$v\,H = v\,C_0 - k\,C_0 \tag{58}$$

The higher the secretion rate the better the approximation. For a k-value of 0.10 and $C_0 = 170$ mN the following differences between eq. (56) and (58) in microequivalents/min will appear:

$v = 0.00$	ml/min—	difference	=	17.0	µeq./min
$v = 0.10$	» —	»	=	8.5	»
$v = 0.20$	» —	»	=	5.8	»
$v = 0.50$	» —	»	=	2.8	»
$v = 1.00$	» —	»	=	1.7	»

When $v > 0.20$ the approximation will be quite justifiable. The actual experimental values lie in general far above this limit.

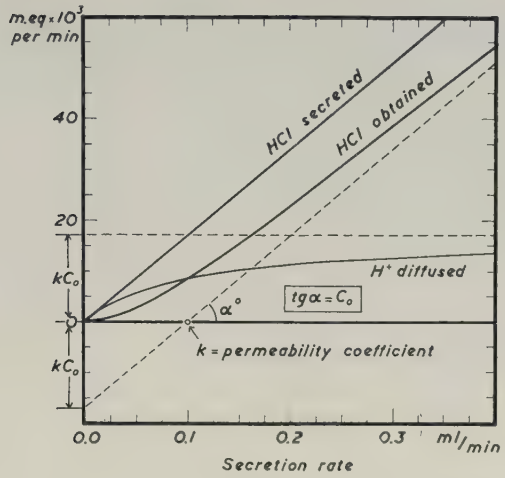


Fig. 30. *The theoretical behaviour of the total amount of HCl obtained in relation to the secretion rate.* It approaches the oblique dotted line which has the mathematical expression $vH = C_0 v - k C_0$. (See text.)

As vH and v are the only variables, the equation (58) is expressed by a *straight line*, where v is the independent and vH the dependent variable, C_0 is the differential factor for the function and kC_0 is the intercept on the vH-axis. The v-intercept will equal k. The meaning of eq. (58) is explained by the *fig. 30* (the oblique dotted line).

Here we ought to call to mind eq. (38) (page 49)

$$H = C_0 \left(1 - \frac{k}{v}\right)$$

which was valid for the case where *the diffusion through the mucous membrane was proportional to the primary acidity itself*, i. e. no mixing of the acid as secreted and the contents of the stomach was possible. Calculating the relation between the total amount and the secretion rate according to this formula the eq. (58) appears *without* the use of any approximation. It was the finding of such a linear relationship between the secretion rate and the output of HCl that made Gray, Bucher & Harman (1941) believe that the relation of acidity to secretion rate followed the hyperbolic curve expressed by eq. (38). (Cf. page 49.)

Experimental data.

The relation between the total acid output and the secretion rate is seen in *fig. 31.* (The results are obtained from dog B.) The points are smoothed with a straight line (freehand) corresponding to the eq.

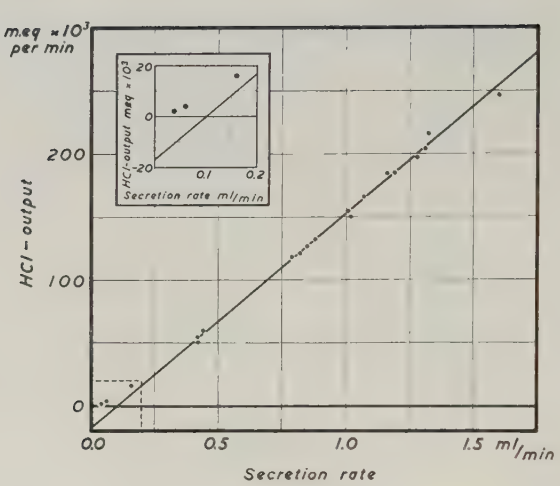


Fig. 31. *The total amount of HCl in relation to the secretion rate* (cf. fig. 30). The dotted square in the lower left-hand corner is enlarged in the inset.

(58). It is seen that the values are very well fitted by this regression line. C_0, i. e. the slope of the line is 170 mN and $k = 0.10$ ml/min. These values agree quite well with those given in Table 8 (page 61).

Only for the lowest secretory rates is there a divergence between the points and the line, indicating that the real experimental curve follows the non-approximated eq. (56) instead of (58). Accordingly *the diffusion from the stomach is proportional to the actual hydrogen ion concentration and not to the primary acidity.*

From this statement it follows that the extrapolation curve given by Gray, Bucher & Harman (1941) for the relation between secretion rate and acidity (cf. p. 49) must be an approximate one, and that the experimental data are in better accordance with the diffusion formula (35), page 48.

CHAPTER 12.

The Chloride Concentration in the Gastric Juice.

Although the acidity has been the most favoured subject for gastric research, the chloride concentration offers just as many problems which are unclear and baffling. A knowledge of the behaviour of the chloride ion would perhaps be valuable in the general discussion of HCl formation.

When reading papers dealing with the problems involved here one very soon finds, that the experimental data are contradictory and vague. Several investigators have found a close relation between the acidity and the chlorides; among them Bolton & Goodhart (1931), Apperly & Crabtree (1931), Hollander (1932), Teorell (1933), Nordenfelt & Teorell (1935), Welin & Frisk (1936), Ihre (1938), Gray & Bucher (1941), Gray, Bucher & Harman (1941), Horstman (1946, 1947); among those who have not Roseman (1907) and Berglund, Johnson & Chang (1935).

With the technique used in this paper there is an opportunity to find the real correlation between the chloride concentration and the secretion rate.

Analogous to the acidity determinations the chloride concentrations are expressed in millinormality (mN).

The chloride concentration in the "primary secretion".

From the results of Linde, Teorell & Öbrink (1947) it may be seen that the Cl values in the "primary secretion" were as high as the acid concentration, and even higher. This is an indication that the acid was secreted as HCl even at the greatest concentrations. As mentioned above (see Chapter 9 page 46) the presence of the isotonic glycine buffer, in which the secreted juice was collected, might have prevented the hypertonicity of such a strong acid. This may possibly be the reason for the high values obtained, and because of the uncertainty at present it seems better to assume a constant primary acidity in the following (cf. page 46).

The "*primary chloride*" will therefore be considered to have a constant concentration of C_0 mN (cf. page 48).

The regulation of the chloride concentration.

The relations between the secretion rate and the "*secondary chloride*" concentration from the present experiments are found in *fig. 32* (the secondary acidity curve is plotted as a dotted line).

It is at once clear that the chloride never followed the low acidity values, but, on the other hand, it showed a certain tendency to decline when the secretion rate was decreasing. As in the case of the acidity, *some reduction of the chloride concentration has occurred though to a lesser extent.*

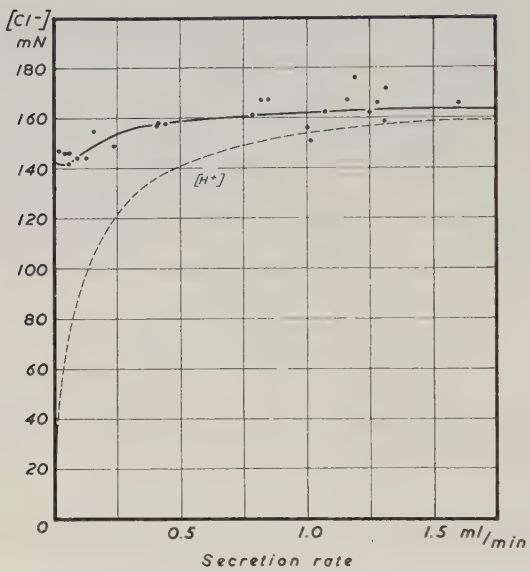


Fig. 32. *The relation between the chloride concentration and the secretion rate.* The experimental date are fitted with eq. (65) by trial and error. $S_0 = 142$ mN; $r = 0.07$ ml/min; $C_0 = 168$ mN; $k = 0.10$ ml/min. (Dog B.)

The three factors acting as acidity reducers may be discussed also when dealing with the chlorides:

1. *Dilution* by a non-acid admixture will of course have the same influence on the chloride concentration as on the acidity, presuming that it is chloride free. However, no diluting fluid in the stomach can be expected to be free from chloride, and this leads us to investigate the possibility of dilution.

2. "*Neutralization*" of chloride is not known to occur in the stomach.

3. *Diffusion* of chloride is very probable, though there are authors who deny any migration of chloride ions through the gastric mucosa — Gilman & Cowgill (1933).

Dilution.

If the main factor in the acidity reduction were an admixture with mucus, this should affect the chloride concentration similarly. The chloride content of the mucus is considered to be 95 mN — Vineberg (1931) — and would consequently diminish the chloride content of the acid. Some higher chloride values are given by Webster (1933) and Baxter (1934).

By *the present technique* it has been possible to obtain low secretion rates with very low acidity concentrations. In these cases the chloride values remained almost constant. This invalidates the theory of dilution by mucus. According to this we should have expected values of about 95—100 mN Cl^- for a secretion rate approaching zero. Another diluting fluid *rich* in chloride would of course afford a possible explanation, but doubt has already been cast on the presence of a diluting secretion for other reasons (cf. page 52).

Diffusion.

There remains diffusion to be discussed. In an instilled volume of HCl (170 mN) in a tied-off cat's stomach the chloride is lowered to 140 –150 mN — Teorell (1933, 1939, 1940). This is also valid for human beings — Ihre (1938), Elliot, Risholm & Öbrink (1942), Kaijser (1943). A diffusion of chloride through the gastric mucosa is also postulated by Shay, Komarow, Siplet & Fels (1946).

The total chloride is the sum of the acid chloride (which is of the same magnitude as the secondary acidity) and the neutral chloride. The latter may be considered as sodium chloride (in reality also other chlorides exist (cf. Gudiksen (1943)). Sodium is proved to be diffusible through the gastric mucosa by experiments with radioactive sodium — Cope, Cohn & Brenizer (1943).

Teorell (1947) made a mathematical evaluation for the diffusion of alkali ions into the stomach contents. He considered the alkali ions as being mainly sodium ions. Denoting the steady state value to which the sodium concentration would approach in a resting stomach as S_0 and the permeability coefficient for sodium as r the following formulae were deduced (v, p and t retain their previous significance, cf. page 48):

$$\textit{increment due to diffusion} = \frac{r\,(S_0 - Na)\,dt}{p + vt} \tag{59}$$

$$\textit{decrement due to dilution} = -\frac{v\,dt}{p + vt} \cdot Na \tag{60}$$

The total concentration changes of sodium will then be

$$\frac{dNa}{dt} = \frac{S_0 r - (r + v)\,Na}{p + vt} \tag{61}$$

which gives

$$Na = \frac{S_0}{\frac{v}{r} + 1}\left[1 - \frac{1}{\left(1 + \frac{vt}{p}\right)^{\frac{r}{v}+1}}\right] \tag{62}$$

If $p = 0$ the expression (62) is reduced to

$$Na = \frac{S_0}{\frac{v}{r} + 1} \tag{63}$$

which expresses a hyperbolic curve starting at S_0 for $v = 0$ and approaching the v-axis as its asymptote for increasing v-values.

If we know the hydrogen as well as the sodium ion concentrations we can calculate the total chloride content (acid chloride + neutral chloride). Thus

$$Cl = H + Na \tag{64}$$

or according to eqs (35) (page 48) and (63)

$$Cl = \frac{C_0}{\frac{k}{v} + 1} + \frac{S_0}{\frac{v}{r} + 1} \tag{65}$$

This eq. represents a curve which starts at S_0 for $v = 0$ and ends at C_0 for $v = \infty$ (cf. fig. 32). Between these two extremities the course of the curve depends on the interrelations between C_0, S_0, k and r.

The relation between chloride concentration and acidity can be calculated from (63) and (64)

$$Cl = H + \frac{S_0}{\frac{v}{r} + 1} \tag{66}$$

Eq. (35) (page 48) can be arranged to give

$$v = \frac{k}{\frac{C_0}{H} - 1} \tag{67}$$

Substituting (67) in (66)

$$Cl = \frac{(k - r)H^2 + r(C_0 - S_0)H + rC_0S_0}{(k - r)H + rC_0} \tag{68}$$

This relation is seen in *fig. 33.*

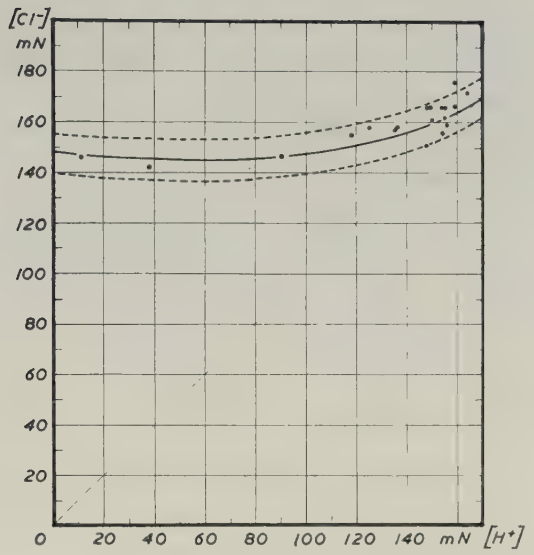


Fig. 33. *The relation between the chloride concentration and the acidity.* The experimental data are fitted with eq. (68) by the method of least squares. $S_0 = 151 \pm 7$ mN; $r = 0.06 \pm 0.01$ ml/min; $C_0 = 168$ mN; $k = 0.10$ ml/min. Standard error of estimate (the dotted lines) $= \pm 8$ mN.

Mathematical evaluation of the experimental data: By using the method of least squares S_0 and r can be determined in the eq. (68) if C_0 and k retain their previous values found in chapter H.

The statistical results are shown in the legend of fig. 33; the regression is represented by the full line and $\pm$ the standard error of estimate by the dotted ones.

The best fit with eq. 65 was, however, found by trial and error. The regression is represented by the full line in fig. 32.

The chloride accumulation in the stomach.

The value of S_0.

At first sight the results seem to give a simple explanation of the chloride concentration in the gastric acid, but there are some details that are rather questionable.

Both in the instillation experiments – Teorell (1933), Ihre (1938) Elliot, Risholm & Öbrink (1942) – and in the present experiments, the steady state value for chloride in a non-secreting stomach is about 140—150 mN. It should be pointed out that the chloride content of

the blood (plasma) is only 100—110 mN (both in man and dog). On account of the Donnan effect a slight difference is to be expected.

It has been suggested — Nielsen (1941)— that the ions in the plasma in man may have the following concentrations:

Cations:		Anions:	
Mg^{++}	2.5 mN	Protein	20 mN
Ca^{++}	5.0 »	Organ. acids	2 »
K^{+}	5.1 »	SO_4^{--}	1 »
Na^{+}	142.4 »	HPO_4^{--}	3 »
		HCO_3^{-}	28 »
		Cl^{-}	101 »
Total	155 mN		155 mN

Assuming all these ions *except the proteins* (20 mN) to be diffusible through the gastric mucosa and denoting the total cations B (basic ions) and the total diffusible anions A (acid ions) the Donnan equilibrium states (cf. Bladergroen (1945), p. 325):

$$\frac{B_{\text{blood}}}{B_{\text{stomach}}} = \frac{A_{\text{stomach}}}{A_{\text{blood}}}$$

or

$$\frac{155}{x} = \frac{x}{135}$$

which makes $x = 145$ mN.

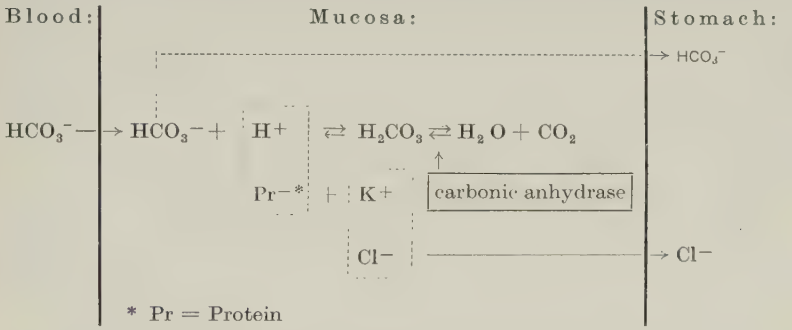
The diffusible anions would thus have a concentration difference between the gastric juice and the plasma of 145—135=10 mN according to the Donnan distribution. The individual anions would then have a concentration in the gastric juice that is $\frac{145}{135}$ times higher than in the plasma. *For the chloride ions that would mean an increase from 100—110 to 107—118 mN and for the bicarbonate ions from 28 to 30 mN.* The bicarbonate ions would, however, be converted to carbon dioxide as soon as any acid secretion started in the stomach. It has, however, been impossible to obtain any considerable CO_2 production in the stomach — Teorell (1933) — which perhaps may indicate that *the bicarbonate ions have never entered the stomach cavity.* The chlorides on the other hand have apparently accumulated in the stomach to a much higher concentration (ca 145 mN) than would be expected from the Donnan equilibrium formulae (107 –118 mN). The following *theory* is proposed as an attempt to explain this phenomenon.

Theoretical.

Davenport (1939) showed one part of the gastric mucosa to be very rich in *carbonic anhydrase*, which he thought to be essential for the acid production (for these theories cf. Davenport (1939, 1940, 1941, 1942), Davenport & Fisher (1940) and Bull & Gray (1945)).

In a recent paper (1946) Davenport himself, however, considers the carbonic anhydrase to be unimportant for the production of the hydrochloric acid.

It may, however, have a definite influence on the diffusion process of the bicarbonate ions according to the following schematical drawing:



If the mucosa is supposed to have the same concentration of protein as the blood plasma — 20 mN — (whether or not this proposition is correct will have no influence on the final result) the bicarbonate entering the mucosa will have the same concentration on both sides of the boundary "blood-mucosa." This equilibrium will be valid also for the Cl^- ions.

When the bicarbonate ions arrive in the carbonic anhydrase "barrier" the following reactions may occur:

$$HCO_3^- + H^+ \rightleftarrows H_2CO_3$$
$$H_2CO_3 \rightleftarrows CO_2 + H_2O.$$

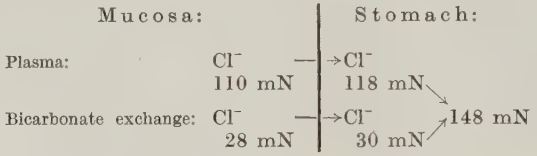
The CO_2 is probably very easily reabsorbed as was found by Linde & Teorell (1947) when they introduced $NaHCO_3$ into a secreting tied-off stomach.

At pH 6.48 (the "apparent" first dissociation constant $= 10^{-6.48}$) 50 per cent of the total CO_2 is present as $H_2CO_3 + CO_2$. Owing to the disappearance of the gas CO_2 and to the catalytic action of carbonic anhydrase the decomposition of H_2CO_3 and the de-ionisation of HCO_3^- will proceed very easily.

The hydrogen ions used in the de-ionisation may be taken from the buffer systems in the mucosa, e. g. the proteins. These are presumably negatively charged at the pH in question.

In the schematical drawing the reaction between the hydrogen ion and the bicarbonate leaves the ions $Protein^-$, K^+, and Cl^-. K^+ and $Protein^-$ (which is indiffusible) interact and to preserve electrical neutrality, Cl^- has to leave the mucosa. *Thus the anion which entered the mucosa (HCO_3^-) has been substituted by the Cl^- ion.* This result is quite analogous to the *chloride shift in the red blood corpuscles.*

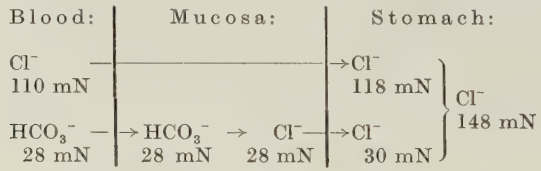
At the boundary "mucosa-stomach" the following situation may then be expected:



One can imagine the chloride ions as originating from two separate sources. *First* the 110 mN from the plasma which have penetrated the mucosa and *secondly* the 28 mN from the mucosa itself which have been substituted for the bicarbonate ions from the plasma.

These chloride concentrations will increase in the stomach according to the Donnan equilibrium. (The mucosa was supposed to have the same amount of indiffusible ions as the plasma — 20 mN —, and the stomach content is considered to be practically free from proteins). Thus the concentration of Cl^- in the stomach would be 118 + 30 = 148 mN.

The net result may be summarized as the following:



The related process makes the gastric mucosa "apparently" impermeable to the bicarbonate ions. From a physical-chemical point of view this impermeability can only be "apparent" because a perfect impermeability to both the proteins (20 mN) and the bicarbonate ions (28 mN) would make only 155 — (20 + 28) = 107 mN anions

permeable. This would give a concentration in the stomach of $\sqrt{155 \times 107} = 129$ mN total anions.

This is not in accordance with the experimental data (145 mN in the stomach), which suggest that only 20 mN are indiffusible (the proteins!). The bicarbonate ions do not give rise to any significant diffusion effects owing to slow diffusibility — Teorell (1935 b, 1937 d) — or to total indiffusibility — Donnan (1911).

As the bicarbonate ions have no diffusion effects and do not enter the stomach they may have been substituted by chloride ions as suggested above.

This theory has to some extent been supported by experimental data.

Experimental.

The essential part of the theory was the reaction

$$H_2CO_3 \rightleftarrows CO_2 + H_2O$$

which was believed to depend on the presence of carbonic anhydrase.

As shown by Davenport (1940) this catalytic action can be inhibited by *thiocyanate*. Doses of 50—200 mg NaSCN were therefore injected intravenously into narcotized cats, into the stomach of which HCl had been instilled. *Fig. 34* shows such an experiment. The stomach was tied off and 5 ml 170 mN HCl were introduced and examined every 15 minutes until the chloride concentration had reached a steady state level (about 145 mN). Then 200 mg thiocyanate were injected. In about 30 minutes the chloride concentration tended to decrease and to approach the chloride concentration in the plasma (109 mN). At the same time the samples began to foam. The foaming was shown not to depend on any increment of proteins in the gastric contents.

This is in accordance with the theory. When the carbonic anhydrase was inhibited the mucosa might have become permeable towards the bicarbonate ions (as indicated by the dotted arrow in the first schematical drawing) which entered the stomach yielding CO_2 on reacting with the instilled HCl. As a result no chloride would substitute the bicarbonate in the stomach, and the chloride concentration would approach the values indicated by the Donnan equilibrium (117 mN).

In order to prove that the changes in the chloride concentration

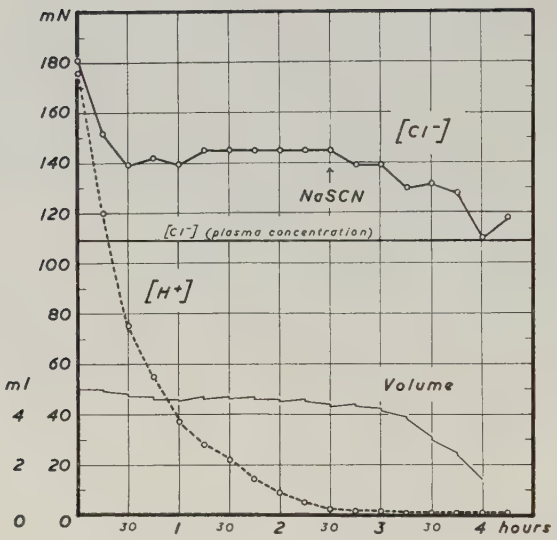


Fig. 34. *Influence of NaSCN on the chloride accumulation in the stomach.* An instillation of 170 mN HCl in a cat's stomach. When a steady state level of the chloride concentration had been reached NaSCN (200 mg) was injected intravenously.

really were accompanied by changes in the diffusibility of the bicarbonate ions, experiments of the following nature were performed (*Table 9*):

Table 9. ***Instillation of 0.20 M glycocol in a stomach of a cat. A new 5.0 ml volume was introduced every period.*** *(* denotes that 200 mg* NaSCN *were injected intravenously at the beginning of a period.)*

Time Minutes	Volume ml		pH	Total acidity mN	Total chloride mM	Bicarbonate mN
	instilled	recovered				
0—15	5.0	5.0	4.2	11	39	3.9
15—30	5.0	5.0	4.4	7	33	0.5
30—45	5.0	5.0	4.2	8	30	3.0
45—60	5.0	5.0	4.3	8	33	4.4
60—90	5.0	5.1	4.0	14	37	0.5
90—105*	5.0	4.7	4.3	8	33	6.7
105—120	5.0	4.9	6.7	3	19	7.1
120—135*	5.0	5.1	7.4	—	21	6.9
135—150	5.0	5.2	7.5	—	19	6.5
150—180*	5.0	5.1	7.7	—	25	11.7
180—195*	5.0	4.7	7.7	—	25	8.9

0.20 M glycocol solution (which does not cause any stimulation or inhibition of the secretion and which does not change its volume in a non-secreting cat's stomach when introduced into it) was kept in the stomach of a cat for 15 or 30 minutes. After this time the bicarbonate content was determined (for method see page 18). The same procedure was then repeated in the same cat after injection of thiocyanate. It was found that the concentration of bicarbonate showed a marked increase after the thiocyanate administration. At the same time the chloride concentration appears to have decreased. The differences in acidity may depend on neutralization by bicarbonate after the thiocyanate administration.

This seems to be further evidence of the correctness of the first part of the theory. The rest of it has, unfortunately, no experimental support beyond the behaviour of the chlorides.

CHAPTER 13.

Total Output of Chloride in Relation to the Secretion Rate.

Theoretical considerations.

According to formula (65) (page 74)

$$Cl = \frac{C_0}{\frac{k}{v} + 1} + \frac{S_0}{\frac{v}{r} + 1}$$

The total amount of Cl will then be

$$v\,Cl = \frac{v\,C_0}{\frac{k}{v} + 1} + \frac{v\,S_0}{\frac{v}{r} + 1} \tag{69}$$

which can be transformed to

$$v\,Cl = v\,C_0 - k\,\frac{C_0}{\frac{k}{v} + 1} + r\,S_0 - r\,\frac{S_0}{\frac{v}{r} + 1} \tag{70}$$

and by substituting (35 and 63) (page 48 and 74)

$$v\,Cl = v\,C_0 - [kH - r\,(S_0 - Na)] \tag{71}$$

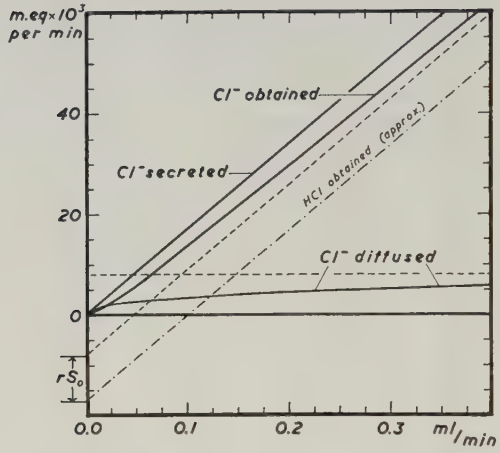


Fig. 35. *The theoretical behaviour of the chloride ions in the stomach.* The Cl^- obtained approaches the interrupted oblique line as its asymptote. rS_0 denotes the neutral chlorides.

The expression vC_0 is the amount of chloride secreted as hydrochloric acid, $r(S_0 - Na)$ is the amount that has diffused into the stomach as neutral chloride and kH the amount which has left the stomach by the diffusion of HCl.

The difference $[kH - r(S_0 - Na)]$ is the net result of the chlorides that have diffused out of the stomach — see *fig. 35*: "*Cl^- diffused.*" In this figure vC_0 is "*Cl^- secreted*" and the vCl is "*Cl^- obtained.*" The line "*HCl obtained (approx)*" is the same as in fig. 30 (page 69).

If $\frac{k}{v}$ and $\frac{r}{v} \ll 1$ the eq. (69) can be changed to

$$vCl = vC_0\left(1 - \frac{k}{v}\right) + rS_0\left(1 - \frac{r}{v}\right) \tag{72}$$

and if $r \leq 1$

$$vCl = vC_0 - (kC_0 - rS_0) \tag{73}$$

This approximated formula differs less from (71) than did the corresponding acidity curve (58) from the formula calculated for it (56). Cf. page 68.

As vCl and v are the only variables in (73) this equation is expressed by a straight line, the slope of which is the same as for (58) ($\operatorname{tg}\alpha = C_0$). The vCl-intersection is $-(kC_0 - rS_0)$ and the distance between the approximated Cl^- and HCl curves in fig. 35 is rS_0 or the total amount *neutral chloride.*

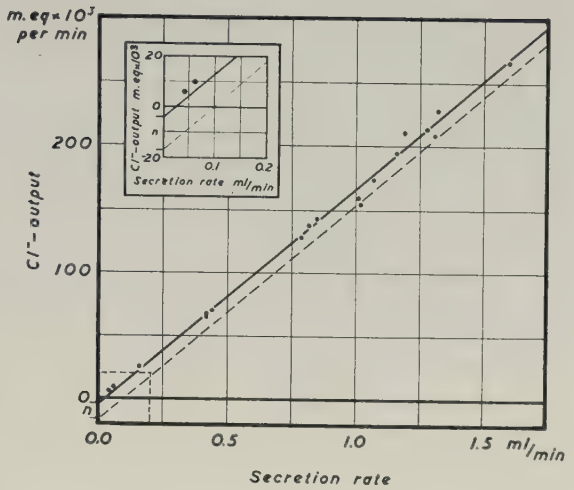


Fig. 36. *Relation between the amount of chloride obtained and the secretion rate.* The dotted square in the lower left-hand corner is enlarged in the inset. The oblique dotted line is the amount HCl obtained (cf. fig. 31) and *n* denotes the neutral chlorides (cf. fig. 35).

Experimental data.

The relation between the total chloride output and the secretion rate is seen in *fig. 36* (dog B). The experimental results are smoothed with a straight line (freehand) corresponding to the eq. (73).

In the figure the approximated HCl curve is also seen. Its distance from the chloride curve is denoted by *n* (neutral chloride).

The points do not agree as well as the corresponding acidity values with the theoretical curve, but are sufficiently well fitted by the line to suggest that the theory may be correct.

The chloride curve is analogous to that given by Gray, Bucher & Harman (1941). They tested their curve statistically and found that it had a somewhat greater angle of inclination than the HCl curve. From this it follows that the amount of neutral chlorides would increase with increasing secretion rates and that the *concentration* of neutral chloride would never approach zero for infinitely large secretion rates.

The difference between their chloride curve and the author's is probably not significant and may be a matter of experimental errors, as can be seen from their statistical values. The slopes of their chloride and acidity curves do not differ statistically. The present curves are parallel owing to the physical-chemical postulates.

CHAPTER 14.

The Excretory Function of the Parietal Cells. Neutral Red Elimination.

Introduction.

Since Fuld (1908) discovered that *neutral red* (N. R.), when poured into the main stomach of a Pavlov dog, appeared in the pouch, many investigations concerning the elimination of this dye (and many others) have appeared. — For a good review of the earlier literature see Kolm, Komarow & Shay (1945). In several clinical studies a *qualitative* relationship between the secretory function of HCl and the elimination capacity of N. R. has been found. In some cases of achylia the dye did not appear at all and in cases of hyperacidity the "appearance time" after an intramuscular or intravenous injection was shorter than normal.

It was shown by Hamperl – see Glaessner & Wittgenstein (1925) — that an accumulation occurred in the parietal cells. Several subsequent experiments have made it appear probable that the dye is exclusively secreted by the parietal cells — Katsch & Kalk (1926), Tejima (1935), Morrison (1938), Kolm, Komarow & Shay (1945). Papers have appeared, however, in which a contrary opinion is maintained as to the place of the excretion. Ingraham and Visscher (1935) have shown that all the dyes eliminated by the gastric mucosa are cationic, i. e. they have a basic chromophoric group.

Besides the stomach, the kidneys and the liver participate in the elimination of neutral red.

In a forthcoming paper by Hallén (1948) some experiments are described which show that in total achylia (pernicious anaemia) the N. R. can be extracted by instillation into the stomach of 150 -170 mN hydrochloric acid though it is not excreted after histamine stimulation. Hallén has also been able to show that N. R., when instilled into the stomach, is reabsorbed by the stomach walls.

Quantitative estimations of the N. R. elimination are rare and give no definite relationship between the N. R. concentration of the gastric juice and the secretion rate of the acidity, though it has been suggested that the concentration of N. R. in the juice runs parallel with the secretion of hydrochloric acid – Kobayashi (1926), Maruno

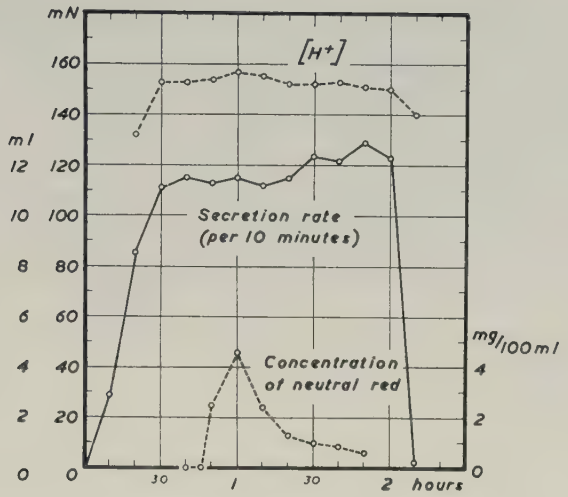


Fig. 37. *The effect of an intramuscular injection of neutral red.* The secretion rate was induced by histamine. At 0^{40} hrs the N.R. was injected.

(1935) and Morrison (1938). Also cf. Kolm, Komarow & Shay (1945).

It was therefore thought most desirable to examine the quantitative relationship between the excretory and the secretory function of the stomach and therefore experiments with neutral red were performed with an improved technique.

Experimental.

The same dogs as in the previous chapters were used (dogs R, S and B). In every experiment the secretion was induced by the continuous intravenous injection of histamine (cf. page 10).

The neutral red was dissolved in water, 1 gram per 100 ml, filtered and sterilized. The final strength of this solution was 0.940 per cent.

In a preliminary experiment the dye was injected intramuscularily with the result given in *fig. 37.* After 6 minutes it appeared in the gastric juice and the concentration rose very quickly to a maximal value within 20 minutes. Then it decreased again. (A slight secretagogue effect could be noticed). It is quite easy to understand that attempts to find the possible interrelation between the secretion and the excretion in this way are extremely difficult. It was therefore necessary to have a constant concentration of neutral red in the blood

during the observations. This could be obtained by injecting the dye intravenously at a constant continuous rate.

The method finally adopted was the following: the injection of the dye was started when a constant secretion rate had been obtained by histamine. It was found necessary to continue the injection of neutral red for at least three hours in order to get a constant colour of the juice. Only the corresponding *steady state values* were used when computing the relationship between the secretion rate and the excretion power.

For the methods of analyzing the concentration of the neutral red in the gastric juice see page 19.

Results.

Constant continuous intravenous injections of N. R. with varying secretion rates.

With the technique described the concentration of neutral red was held constant by the continuous intravenous injection during many experiments, but the injection rate of histamine was varied, thus causing varying secretion rates. A typical experiment of this kind is seen in *fig. 38.*

The result obtained by plotting the steady state values from several similar experiments in a coordinate system is shown in *fig. 39* (dog B).

The concentration of N. R. in the juice was higher the lower the secretion rate. The same result was obtained in all the dogs used.

The *total amount* of N. R. eliminated through the pouch was calculated by multiplying the concentration by the volume secreted and gave the relation to the secretion rate as shown in *fig. 40.* This seems to be an almost linear relationship, so that *the total amount eliminated by the gastric juice increased with increasing secretion rates.*

It might be of interest to compare the amount injected with the amount eliminated by the stomach.

In the experiments described 37.6 mg/hour have been injected and at the highest secretion rate (1.42 ml/min) 16.67 μg/min were eliminated (cf. fig. 40). This makes $\frac{16.67 \times 60}{1000} \approx 1$ mg/hour eliminated. Considering the pouch to be about 1/4 of the entire stomach, 4 mg neutral red were eliminated per hour. That makes about 10 % of the amount injected. It ought to be remembered that the computations

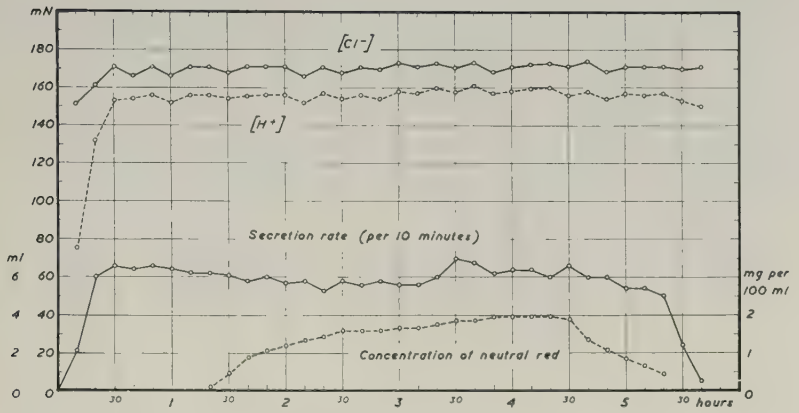


Fig. 38. *A typical experiment with continuous intravenous injection of histamine and neutral red.* When the secretion rate was constant the injection of N.R. was started. The injection of N.R. was stopped at 4^{30} hrs and that of histamine at 5^{20} hrs.

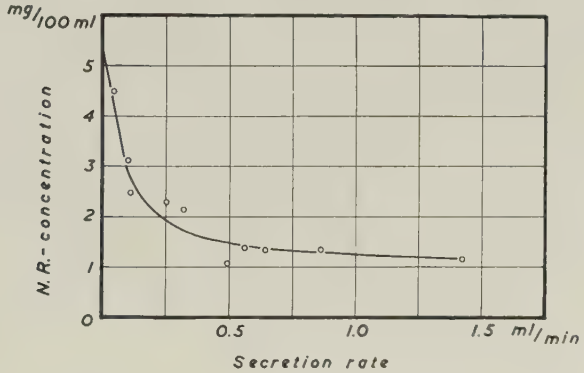


Fig. 39. *The relation between the concentration of N.R. in the gastric juice and the secretion rate.* N.R. was injected at a constant rate (37.6 mg/hour). Every value represents a steady state level.

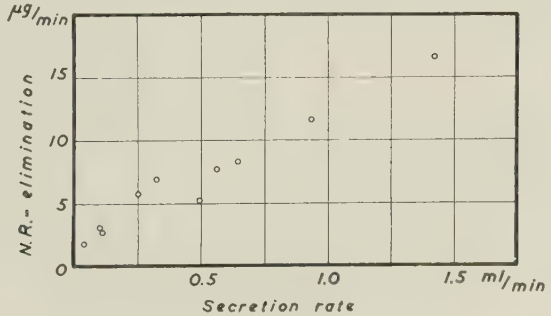


Fig. 40. *The relation between the total amount of N.R. eliminated by the gastric juice and the secretion rate.* The values are obtained from fig. 39.

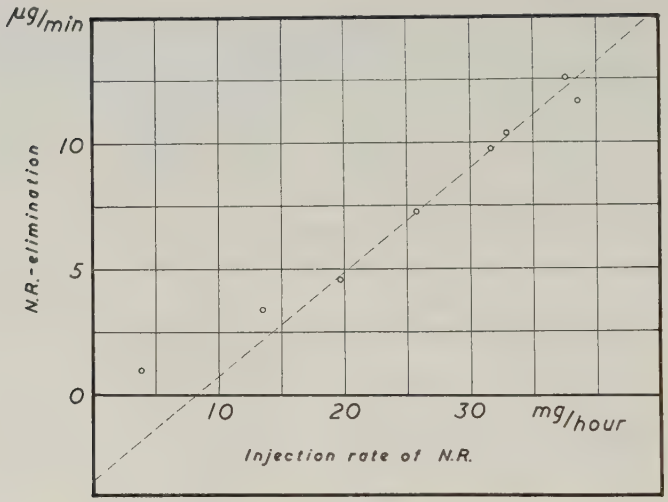


Fig. 41. *The relation between the injection of N.R. and the amount eliminated by the gastric juice.* The secretion rate was kept constant (cf. Table 10). Every value is obtained from a steady state level. The freehand dotted line gives an intercept on the ordinate which may indicate a resorption of N.R. from the gastric pouch.

were made during steady state conditions, i. e. the amount eliminated equals the amount injected. (As 37.6 mg/hour were injected the same amount is considered to be eliminated.) Thus *the stomach is responsible for at most about 10 % of the total elimination of neutral red.*

This fact may be discussed as it invalidates to some extent the suggestion made above that the injection rate of neutral red is directly proportional to the steady state value of the concentration in the blood. With an increased secretion rate the elimination was increased (fig. 40). The theoretical basis for a straight line relation between the injection rate and the steady state concentration was, however, the constancy of all diffusion and elimination constants (cf. chapter 4 page 20).

It is considered, however, that the changes in the elimination will only have a slight influence on the concentration in the blood.

Influence of the blood concentration of neutral red upon the elimination by the gastric mucosa.

As is seen from the foregoing, the secretion rate has a definite influence on the elimination capacity of N. R. by the gastric mucosa. In order to investigate the relation between the blood concentration of N. R. and the elimination it is therefore essential to have a constant

Table 10. *The influence of different injection rates of neutral red on the elimination of the dye in the gastric juice. All values are obtained from steady state levels.*

Injection rate of N. R. mg/hour	Secretion rate of gastric juice ml/min	Acidity mN	NR in the gastric juice	
			Concentration mg/100 ml	Amount µg/min
3.9	0.57	135	0.18	1.02
13.5	0.63	146	0.54	3.38
19.7	0.67	153	0.69	4.59
25.7	0.60	151	1.22	7.32
31.6	0.66	153	1.48	9.77
32.9	0.55	153	1.90	10.36
37.6	0.67	168	1.80	12.06
38.5	0.60	159	1.95	11.70

and identical secretory rate in all experiments. If a large secretion rate is chosen small changes will, however, have but a slight influence as seen from fig. 39.

This was done in dog R and in these experiments the secretion rates varied between 0.55—0.67 ml/min.

The injection rates of N. R. can be considered as proportional to the steady state values of the blood concentration.

The results are shown in *Table 10* and *fig. 41*, where the total amount (µg/min) eliminated by the pouch is plotted against the injection rate of N. R. (= the blood concentration of N. R.).

The points lie on a curve which, at least for the higher values of injection rates, seems to be a straight line. This is shown as the dotted line in the figure.

It is quite clear from this curve that *the rate of elimination will be dependant on the concentration of the dye in the blood.* This is the reason why the curve in fig. 37 has its particular shape after an intramuscular injection of N. R., as the blood concentration of N. R. probably has a similar time-concentration relation.

Attempts have been made to analyze the concentration of N. R. in the blood but, even during the largest injection rates of N. R., no N. R. could be identified in the blood. Acidifying, oxidation with ceric sulphate in an acid solution, extraction with ether-alcohol mixtures gave all the same negative results. This indicated that an enormous accumulation had occurred in the gastric mucosa, as described by Kolm, Komarow & Shay (1945) and others.

Discussion.

With the aid of the continuous intravenous injection technique, both the concentration of neutral red in the blood and the secretion rate of gastric acid were held at constant steady state levels. This made it possible to study the relation between the secretion rate and the elimination of the dye in the stomach as well as the influence of the blood concentration on this elimination.

The interesting relationship between the concentration of N. R. in the gastric juice and the secretion rate may perhaps be explained by different mechanisms.

A probable one may be that N. R. is accumulated to a high concentration in the mucosa cells (the parietal cells) while the *intercellular* fluid also contains some dye but at a significantly lower concentration. Such a difference in the concentration between cells and surroundings is shown to exist in an aqueous solution of baker's yeast — COLLANDER and ÄYRÄPÄÄ (1947). The water for the hydrochloric acid secretion is supposed to be taken from the intercellular fluid thus giving a gastric secretion which is mixed up with the dye from the beginning. While passing through the intra- and/or intercellular canaliculae the secretion (now with a low pH) will take up more N. R. from the cells by a diffusion process. This diffusion may be more significant at small secretion rates. Thus the relation between the secretion rate and the concentration of N. R. in the juice will be that found experimentally.

The processes can be described by the regression line

$$C = C_0 - (C_0 - C_\infty)\, e^{-\frac{k}{v}} \tag{74}$$

which is shown in fig. 39. Here C is the concentration of N. R. in the juice; v the secretion rate; the constants C_0 and C_∞ the concentration in the juice at infinitely small and high secretion rates; k a constant.

This regression line is quite well fitted by the experimental data as seen in fig. 39. Whether or not the theoretical basis is correct the equation is a good expression of the relationship between the secretion rate and the concentration of neutral red in the gastric juice.

It may therefore be justifiable to use this equation in fig. 40 as well. The total amount secreted will then be

$$v\,C = v\,C_0 - v\,(C_0 - C_\infty)\, e^{-\frac{k}{v}} \tag{75}$$

From chapter 10 the relation between the secondary acidity and the secretion rate is known to have a shape quite different from the N. R. concentration just described. That means that *there is no parallelism between the secondary acidity and the concentration of neutral red.*

One should keep in mind the relationship between the secretion rate and the primary acidity (fig. 21, page 45). The resemblance between the two curves may or may not be determined by a common factor.

The elimination depends not only on the secretion rate, but also on the concentration of the dye in the blood and tissue (fig. 41). It can be deduced from the theoretical basis given above, and it is quite possible and plausible, that the amount eliminated may be directly proportional to the blood and tissue concentration of the dye. This would give a straight line relation passing through the origin.

Any deviation from such an expected curve that may be shown by the experimental data in fig. 41 might be a result of back diffusion of neutral red through the gastric mucosa. This would be in accordance with the results obtained by HALLÉN (1948).

The practical consequences of the above may be that the clinical use of neutral red is limited to *qualitative* experiments based on appearance or absence or on an "appearance time" of the dye, unless a laborious method like the present one is used.

Conclusions: The elimination of neutral red was found to be dependant on both the secretion rate and the concentration of the dye in the blood. The secondary acidity does not run parallel with the concentration of neutral red in the gastric juice.

Intramuscular (or massive intravenous) injections do not afford any possibility of investigating the correlations between the elimination and any properties of the parietal secretion.

The eliminated dye is supposed to come both from the intercellular fluid in the course of the process of secretion of HCl and from the cells — which are supposed to accumulate the dye — by a diffusion process.

CHAPTER 15.

Influence of Enterogastrone on the Parietal Secretion.

Historical.

Since more than fifty years many authors have reported that fat given to an animal depressed the secretion of gastric acid — Ewald & Boas (1886), Chischin (1894), Wirschubski (1900), Fermi (1901), Walko (1903), Kasanki (1903). Fat was also used against hyperacidity and ulcers — Cohnheim (1904), Ebstein (1904).

Szokolow (1904) separating the duodenum from the stomach in a dog, found that it was only the presence of fat in the duodenum which suppressed the secretion of HCl in the stomach. The effect was not abolished by vagotomy — Lim, Ivy & McCarthy (1925) — and was found even in a completely denervated gastric pouch — Feng, Hou & Lim (1929).

On the basis of these experiments it has been suggested that the presence of fat in the duodenum causes it to secrete an active principle which has the effect of diminishing gastric secretion. Kosaka & Lim (1930) suggested the name *enterogastrone* for this active principle, which could be extracted from the mucosa of the small intestine.

Important papers concerning enterogastrone have appeared by Ivy and his co-workers. Gray, Bradley & Ivy (1937) developed a method of assay for a purified enterogastrone preparation. Hands, Greengard, Preston, Fauley & Ivy (1942) discovered that *enterogastrone prevented experimental jejunal ulcers* in dogs. This stimulated further work in this field — Hands, Greengard, Fauley & Ivy (1943), Grossman, Greengard, Dutton & Woolley (1944), Ivy (1944), Grossman & Ivy (1946). Also cf. the paper about pepsin secretion and enterogastrone by Grossman, Greengard, Woolley & Ivy (1944).

Enterogastrone preparations have also been used in the treatment of ulcers in man with good results by Greengard, Atkinson, Grossman & Ivy (1946).

The chemical properties of purified enterogastrone.

Öbrink (1947 a) found that the active principle in *enterogastrone preparations has no or at least a very slight electrophoretic mobility between*

pH 2.06 and 7.42. WINBERG (1947) reported that the active principle in the same preparation was *easy to dialyse* through a cellophane membrane. He made use of this property and purified the preparation by dialysis. In the dialysate a very active concentrate was recovered.

This dialyzed enterogastrone could not be inactivated by the action of pepsin or trypsin — WINBERG & ÖBRINK (1948) – which led to the conclusion that it is *probably not a protein.* However, the nitrogen content is 15 %, which makes these results unexpected or may indicate that the preparation is still very impure. Nevertheless its biological activity is extremely high.

The physiological properties of enterogastrone.

Several experiments have been performed on the dogs using this enterogastrone preparation (Eg)[1]. In this paper the main question is in what sense it has any influence on the parietal secretion. Besides this there is, however, one interesting thing which ought to be reported here in a preliminary way.

In most experiments the Eg was injected intravenously in a massive dose. 50 mg were enough to give the following symptoms:

If the injection is made too rapidly (e. g. in about 30 seconds) the dogs become *unconscious* and develop *convulsions.* This attack starts in about one minute after the beginning of the injection and lasts for about five minutes. Afterwards the dog is rather tired. During the attack the bladder is emptied and defaecation sometimes occurs.

If, at the start of the attack or during it, some mls of a 5 % glucose solution is injected intravenously the dog's recovery is immediate and complete.

There are many experiments which will have been carried out in this field before any conclusions can be drawn, but the phenomenon seems sufficiently interesting to deserve this preliminary report.

The influence of Eg on the parietal secretion.

In chapter 6 it was shown that there was a close relation between stimulus and secretion. When an inhibitory (or a stimulating) substance is to be tested on the dog with a pouch it is essential to stimulate the mucosa submaximally. This was pointed out by

[1] The preparations have been made by Dr WINBERG, *A. B. Astra*, Södertälje, Sweden.

Öbrink (1946), and is easily understood from fig. 14 (page 33). When the secretion rate is maximal, a stimulating agent may have no influence and the same may be true for inhibiting substances. It is therefore necessary to have a secretion which corresponds to the ascending part of fig. 14. In the present enterogastrone experiments this point has been under complete control.

As enterogastrone may be a substance which acts in the physiological regulation of the parietal secretion, a thorough investigation of its rôle seems justifiable.

The questions arising are:

What is the influence of enterogastrone on

1. the *histamine concentration* in the plasma,
2. the *secretion rate* in histamine-induced gastric secretion,
3. the *acidity* of this secretion,
4. the *chloride concentration* in it,
5. the *permeability properties* of the gastric mucosa and
6. the *neutral red excretion* by the parietal cells?

1. *Influence on histamine concentration in the plasma.*

From chapter 4 the relations between the injection rate of histamine and the plasma concentration are known. In chapter 6 page 30 this knowledge is used to obtain the relation between the concentration of histamine in plasma and the secretion rate.

By using Eg we have the possibility of depressing the gastric secretion, and the question now is whether or not this depression is dependant on a decrease in the concentration of histamine (histaminase effect).

In *Table 11* this question is answered. When the histamine injection had resulted in a constant secretion rate, 50 mg Eg-preparation were given intravenously in about 15 seconds. (The attack described above occurred.) The secretion decreased and disappeared in spite of the continued histamine stimulation. The histamine analyses in plasma samples before and after the Eg injection showed no significant difference. (Because of shortness of material only one experiment could be made. Also cf. the error of the method of analyzing for histamine, page 16).

It may be stated that enterogastrone probably does not inhibit the gastric secretion by destroying the histamine in the blood.

Table 11. *The effect of enterogastrone on the secretion rate and the plasma concentration of histamine.*

Injection rate of histamine mg/hour	Secretion rate ml/min	Histamine in plasma µg/100 ml
Before enterogastrone injection		
1.8	0.62	9.9
After enterogastrone injection		
1.8	0.03	9.0

2. *Influence on the histamine-induced gastric secretion rate.*

As seen above the injection of Eg results in a decrease in the gastric secretion rate. This is also seen from *fig. 42* and *44*.

The present author has never observed a refractory state of the dogs towards enterogastrone such as was reported by Gray & Wieczorowski (1939) and by Friedman & Sandweiss (1946) when they tested the gastric depressant in urine, *urogastrone*.

The effect has appeared at different times after the injection from a few minutes to 45 minutes. The longest time lag (45 minutes) was obtained with the most active preparations obtained from dialysates, but the significance of the time lag is quite unknown.

The effect has in many cases amounted to an absolute abolition of the secretion (as seen in fig. 42), and the effect has lasted from 3—7 hours.

In forthcoming experiments the correlation between the inhibitory effect and the dose of enterogastrone will be studied.

3. *Influence on the acidity of the gastric secretion.*

In fig. 42 a typical experiment with an effective enterogastrone preparation shows the behaviour of the acidity.

When the secretion rate decreases the acidity follows but shows a considerable time lag. This time lag may, however, depend on the impossibility of emptying the pouch completely, and the resulting formation of a "lake" around the rubber tube (fig. 27) which was described in chapter 10 page 63.

A "lake" containing 4 ml such as has been found in the experiments

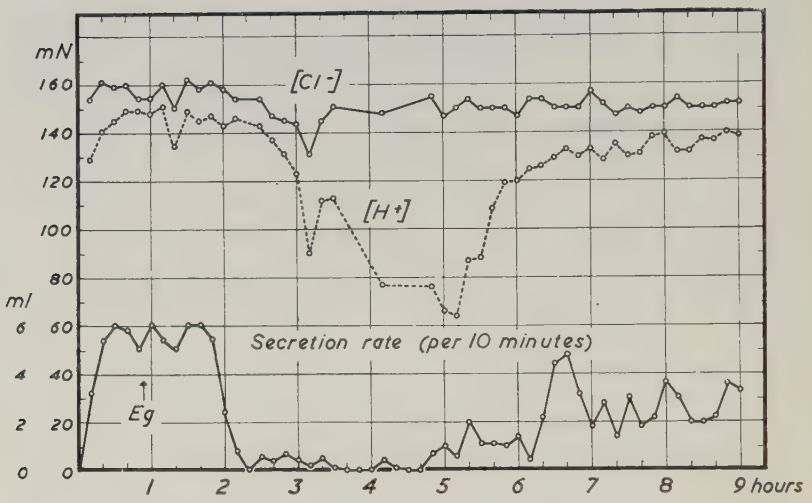


Fig. 42. *The effect of enterogastrone (Eg) on the gastric secretion.*

described on page 63, would explain the behaviour of the acidity curve.

It is most likely that all changes in the acidity are due to changes in the secretion rate, and that *there is no specific action by enterogastrone on the acidity.*

4. *Influence on the chloride concentration of the gastric secretion.*

As we know the relation between the chloride concentration and the secretion rate, we do not expect to find any considerable changes in the chlorides after the administration of Eg, provided that this has no *specific* action on the kinetics of the chloride secretion.

Fig. 42 shows that no such action is obtained. Therefore *enterogastrone has no direct influence on the chloride concentration in the gastric juice.*

5. *Influence on the permeability properties of the gastric mucosa.*

The decrease in acidity following the instillation of HCl has already been described (page 66). This reduction in acidity is supposed to be caused by an interchange of sodium and hydrogen ions across a diffusion membrane, chloride ions being the main anions on both sides of this membrane. It is of interest in the present connection to see whether this process is affected by enterogastrone.

Such instillation experiments have been performed on cats. *Fig. 43*

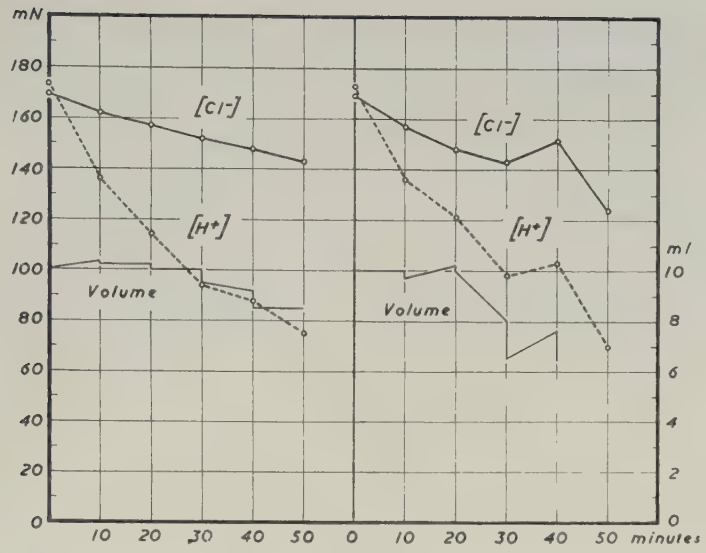


Fig. 43. *The acidity reduction of an instilled volume HCl before and after administration of enterogastrone.*

shows that the course of the curves is in agreement with normal instillation experiments, indicating that *enterogastrone seems to have no influence on the permeability of the gastric mucosa toward hydrochloric acid.*

6. *Influence on the neutral red excretion by the gastric mucosa.*

In chapter 14 page 84 ff. the relation between the excretion of neutral red (N. R.) and the secretion rate was considered. If the concentration of *the dye was held at a constant level in the blood plasma* by a continuous intravenous injection, the excretion of the dye was dependent on the rate of secretion as shown by fig. 39, page 87. The lower the secretion rate the higher the concentration of neutral red in the gastric juice.

In *fig. 44* the influence of enterogastrone on this special property of the parietal cells is considered. The histamine injection is started at 0 hours. When the secretion rate has become constant the injection of neutral red is begun (2^{56} hrs). When the colour of the secreted juice seems to be constant, enterogastrone is administered intravenously (4^{20} hrs) and secretory inhibition results. The injection of N. R. is stopped at 5^{36} hrs and that of histamine at 6^{20} hrs.

After the administration of enterogastrone the secretory rate decreases to about 60 %. The N. R. concentration increases from 4

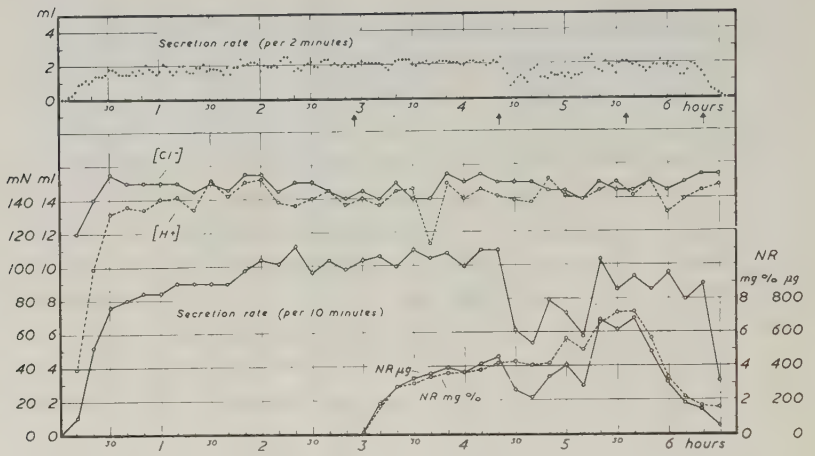


Fig. 44. *The influence of enterogastrone on neutral red excretion.* At 2^{56} injection of N.R. is started, at 4^{20} Eg is injected, at 5^{36} and 6^{20} the injections of neutral red and histamine respectively are ended.

to 7 mg/100 ml after a time lag which may be explained by the "lake" (page 63). This increase in the dye concentration is, however, expected from fig. 39 page 87.

It is therefore most probable that *enterogastrone has no specific influence on the excretory function of the parietal cells.*

Conclusions: — The above observations have shown that enterogastrone only affects the volume of secretion produced by the parietal cells. This effect is probably not due to any histaminase effect of enterogastrone.

No specific action on the acidity, chloride concentration or excretory function could be found after enterogastrone administration, nor did the permeability of the mucosa show any changes in respect to hydrochloric acid.

General Summary.

The Purpose of the Present Investigation.

The intention was to study the parietal cell secretion in the stomach from a kinetic point of view. Chapter 1 (page 7).

Procedure.

The investigations were carried out on Heidenhain dogs and, in some experiments, on cats.

Histamine was used as a stimulating agent and was given by continuous intravenous injection using a specially constructed injection apparatus which is described.

An improved technique for collecting the gastric juice was used. Chapter 2 (page 8).

Methods of Analysis.

All the methods employed for the analyses are described. They include the determinations of pH, acidity, chloride, pepsin, nitrogen, reducing power, calcium, bicarbonate, phenol red and neutral red in the gastric contents and of histamine in the plasma. Chapter 3 (page 14).

The Histamine Distribution in the Body.

The kinetics of the distribution of a drug administered to the body by means of a subcutaneous or continuous intravenous injection were discussed on the basis of theoretical considerations.

Experiments showed that — in agreement with the theoretical considerations – the histamine concentration in plasma was proportional to the injection rate of histamine. Chapter 4 (page 19).

Time-secretion Relation after Continuous Intravenous Injection of Histamine.

The secretion could be held at a constant rate by the stimulation technique employed. Any changes in the injection rate were followed by similar changes in the secretion rate. Under special conditions an *apparent* independence between stimulus and secretion could be obtained. Chapter 5 (page 26).

Relation between Histamine Concentration and Rate of Secretion. Concentration-action Curves.

It was suggested that the relation between the injection rate of histamine (r) and the secretion rate (v) could be described by the expression

$$v = a(1 - e^{-kr}),$$

where a = the maximal secretion rate; k = constant.

The concentration-action curve was treated according to the method given by Gaddum. Chapter 6 (page 30).

The Secretion of Gastric Juice after Subcutaneous Injections of Histamine.

The relation between the dose of histamine and the time-secretion curve was discussed. A close relation to the maximal secretion rate or to the total amount secreted was found only when small doses of histamine were used.

The "continuous histamine test," i. e. frequently repeated subcutaneous injections of histamine ,was discussed. CHAPTER 7 (page 37).

The Minimal Effective Dose of Histamine Producing Gastric Acid.

It was suggested that there exists no threshold value for the action of histamine in stimulating the gastric secretion. CHAPTER 8 (page 41).

On the Primary Acidity.

Reference was made to a recent investigation which suggested the possibility that the primary acidity increased with decreasing secretory rates. CHAPTER 9 (page 43).

Regulation of the Acidity.

Three possibilities of explaining the acidity regulation were discussed: *dilution*, *neutralization* and *diffusion*.

The experimental data indicated that in a Heidenhain pouch stimulated with histamine, the acidity regulation was mainly due to diffusion processes according to the "diffusion theory."

Some inconsistencies of the theory were explained by the fact that the gastric pouch could never be completely emptied but contained a remaining volume, which was thought to interfere with the relation between the acidity and the secretion rate. CHAPTER 10 (page 46).

Total Output of Hydrochloric Acid in Relation to the Secretion Rate.

A simple relation between the secretion rate and the total output of HCl was found both theoretically and experimentally. The relation was considered to be a net result of secretion and diffusion. CHAPTER 11 (page 67).

The Chloride Concentration in the Gastric Juice.

The concentration of chloride in the gastric juice was found to be in accordance with the diffusion theory.

The chloride accumulation in the stomach was discussed and a new theory was proposed as an attempt to explain the phenomenon. The

theory assumed that bicarbonate ions of the plasma were "indiffusible" through the gastric mucosa due to the presence of carbonic anhydrase. In the mucosa the bicarbonate ions were substituted by chloride ions which entered the stomach. — Some experiments with sodium thiocyanate seemed to support the theory. Chapter 12 (page 70).

Total Output of Chloride in Relation to the Secretion Rate.

A simple relation between the secretion rate and the total amount of Cl^- was found theoretically and experimentally on the basis of the "diffusion theory." Chapter 13 (page 81).

The Excretory Function of the Parietal Cells. Neutral Red Elimination.

It was considered that continuous intravenous injection of neutral red would maintain the concentration of the dye in the blood at a constant level. Under such conditions the relation between the secretion rate and the neutral red concentration in the gastric juice was observed. The lower the secretion rate the higher the concentration of the dye in the gastric juice.

With the same technique the relation between the blood concentration of neutral red and the elimination was also studied. The resulting curves indicated that reabsorption of the dye possibly occurred. The results were discussed. Chapter 14 (page 84).

Influence of Enterogastrone on the Parietal Secretion.

An active enterogastrone preparation was found to influence the secretion rate alone and had no *specific* action on the acidity, chloride concentration or neutral red elimination. The depressing effect on the secretion rate was probably not due to any histaminase effect of the enterogastrone. Chapter 15 (page 92).

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References.

Apperly, F. L. and M. G. Crabtree, J. Physiol. 1931, *73*, 331.

Babkin, B. P., *Secretory Mechanism of the Digestive Glands*, New York 1944.

Babkin, B. P., C. O. Hebb and L. Kreuger, Quart. J. exp. Physiol. 1941, *31*, 63.

Bandes, J., F. Hollander and J. Glickstein, Amer. J. Physiol. 1940, *131*, 470.

Baxter, S. G., Amer. J. Dig. Dis. 1934, *1*, 36.

Beaumont, W., *Experiments and Observations on the Gastric Juice and the Physiology of Digestion*, Plattsburg 1833.

Berglund, H., R. Johnson and H. C. Chang, Acta Med. Scand. 1935, *86*, 269.

Björkman, G., Å. Nordén and B. Uvnäs, Acta Physiol. Scand. 1943, *6*, 108.

Bladergroen, W., *Physikalische Chemie in Medizin und Biologie*, Basel 1945.

Boldyreff, W. N., Pflüg. Arch. ges. Physiol. 1907, *121*, 13.

—— Ergebn. Physiol. 1911, *11*, 121.

—— Quart. J. exp. Physiol. 1914, *8*, 1.

—— Amer. J. Physiol. Proc. 1933, *105*, 9.

—— Acta Med. Scand. 1934, *82*, 111.

Bolton, C. and G. W. Goodhart, J. Physiol. 1931, *73*, 115.

Bucher, G. R., A. C. Ivy and J. S. Gray, Amer. J. Physiol. 1941, *132*, 698.

Bull, H. B. and J. S. Gray, Gastroenterology 1945, *4*, 175.

Carlson, A. J., Physiol. Reviews 1923, *3*, 1.

Chischin, P., Jahresbericht ü. d. Thier-Chemie 1894, *24*, 347.

Code, C. F., J. Physiol. 1937, *89*, 257.

Cohnheim, P., Zeitschr. f. Klin. Mediz. 1904, *52*, 110.

Collander, R. and T. Äyräpää, Acta Physiol. Scand. 1947, *14*, 171.

Colldahl, H., C. G. Holmberg and C.-B. Laurell, Acta Physiol. Scand. 1946, *12*, 1.

Conway, E. J. and T. Brady, Nature 1947, *159*, 137.

Cope, O., H. Blatt and M. R. Ball, J. Clin. Invest. 1943, *22*, 111.

Cope O., W. E. Cohn and A. G. Brenizer Jr., J. Clin. Invest. 1943, *22*, 103.

Dale, H. H. and P. P. Laidlaw, J. Physiol. 1919, *52*, 355.

Davenport, H. W., J. Physiol. 1939, *97*, 32.

—— Amer. J. Physiol. 1940, *129*, 505.

—— Amer. J. Physiol. Proc. 1941, *133*, 257.

—— Amer. J. Dig. Dis. 1942, *9*, 416.

— Gastroenterology 1943 a, *1*, 383.

— Gastroenterology 1943 b, *1*, 1055.

—— Gastroenterology 1946, *7*, 374.

Davenport, H. W. and R. B. Fischer, Amer. J. Physiol. 1940, *131*, 165.

Davies, R. E., N. M. Longmuir and E. E. Crane, Nature 1947, *159*, 468.

Donnan, F. G., Z. Elektrochem. 1911, *17*, 572.

Ebstein, W., Deutsche Mediz. Wochenschr. 1904, *30*, 1749.

Edkins, J. S., J. Physiol. 1906, *34*, 183.

Elliot, A., L. Risholm and K. J. Öbrink, Acta Med. Scand. 1942, *110*, 267.

Emmelin, N., Acta Physiol. Scand. 1945, *11*, Suppl 34.
Emmelin, N. and G. S. Kahlson, Acta Physiol. Scand. 1944, *8*, 289.
Emmelin, N., G. Kahlson and F. Wicksell, Acta Physiol. Scand. 1941, *2*, 123.
Engeström, T., Acta Med. Scand. 1935, *85*, Suppl. 66.
Ewald, C. A. and J. Boas, Virchow Arch. f. Anat. 1886, *104*, 271.
Ezekiel, M., *Methods of Correlation analysis*, New York 1945.
Feldberg, W. and E. Schilf, *Histamin* (Monograph aus dem Gesamtgebiet der Physiologie der Pflanzen und der Tiere), Berlin 1930.
Feng, T. P., H. C. Hou and R. K. S. Lim, Chin. J. Physiol. 1929, *3*, 371.
Fermi, C., Arch. Physiol. Suppl. 1901, p. 1.
Flensborg, E. W., *Ventrikelsekretionen hos normale og syge spæd- og smaaborn*, Roskilde 1944. (Danish.)
Folin, O. and H. Malmros, J. Biol. Chem. 1929, *83*, 115.
Friedman, H. F. and D. J. Sandweiss, Amer. J. Dig. Dis. 1946, *13*, 108.
Fuld, E., Münchener Med. Wochenschr. 1908, *55*, 2264.
Gaddum, J. H., Med. Res. Council, Spec. Report *183*, 1933.
Gilman, A. and G. R. Cowgill, Amer. J. Physiol. 1933, *103*, 143.
Giraud-Costa and Gayral, C. r. Soc. Biol. Paris 1940, *134*, 88.
Glaessner, K. and H. Wittgenstein, Arch. f. Verdauungskrankh. 1925, *34*, 303.
Grant, R., Amer. J. Physiol. 1941, *132*, 467.
—— Amer. J. Physiol. 1942, *135*, 496.
—— Amer. J. Physiol. 1944, *141*, 496.
Gray, J. S., Gastroenterology 1943, *1*, 390.
Gray, J. S., W. B. Bradley and A. C. Ivy, Amer. J. Physiol. 1937, *118*, 463.
Gray, J. S. and G. R. Bucher, Amer. J. Physiol. 1941, *133*, 542.
Gray, J. S., G. R. Bucher and H. H. Harman, Amer. J. Physiol. 1941, *132*, 504.
Gray, J. S. and E. Wieczorowski, Proc. Soc. exp. Biol. Med. 1939, *40*, 324.
Greengard, H., A. J. Atkinson, M. I. Grossman and A. C. Ivy, Gastroenterology 1946, *7*, 625.
Greengard, H., I. F. Stein and A. C. Ivy, Amer. J. Physiol. 1941, *132*, 305.
Grossman, M. I., H. Greengard, D. F. Dutton and J. R. Wolley, Gastroenterology 1944, *2*, 437.
Grossman, M. I., H. Greengard, J. R. Wolley and A. C. Ivy, Amer. J. Physiol. 1944, *141*, 2.
Grossman, M. I. and A. C. Ivy, Fed. Proc. 1946, *5*, 37.
Grossman, M. I., Abstr. XVII. Internat. Physiol. Congr. 1947, 297.
Gudiksen, E., Acta Physiol. Scand. 1943, *5*, 39.
Gutowski, B., Soc. Biol. Paris 1924 a, *91*, 1346.
—— Soc. Biol. Paris 1924 b, *91*, 1349.
Hallén, L., to be published.
Hands, A. P., H. Greengard, G. B. Fauley and A. C. Ivy, Fed. Proc. 1943, *2*, 18.
Hands, A. P., H. Greengard, F. W. Preston, G. B. Fauley and A. C. Ivy, Endocrinology 1942, *30*, 905.
Harvey, B. C. H. and R. R. Bensley, Biol. Bull. 1912, *23*, 225.

Heidenhain, R., Pflüg. Arch. ges. Physiol. 1878, *18*, 169.
—— Pflüg. Arch. ges. Physiol. 1879, *19*, 148.
Hollander, F., Proc. Soc. exp. Biol. Med. 1932, *29*, 640.
—— J. Biol. Chem. 1934, *104*, 33.
—— Amer. J. Dig. Dis. 1938, *5*, 364.
—— Gastroenterology 1943, *1*, 401.
Hollander, F. and G. R. Cowgill, J. Biol. Chem. 1931, *91*, 151.
Horstmann, P., Nordisk Medicin 1946, *32*, 2398.
—— Acta Physiol. Scand. 1947, *14*, 27.
Ihre, B., Acta Med. Scand. 1938, Suppl. 95 (also in Oxford Medical Publ. 1939).
Ingraham, R. C. and M. B. Visscher, J. gen. Physiol. 1935, *18*, 695.
Ivy, A. C., Gastroenterology 1944, *3*, 443.
Ivy, A. C. and J. I. Farrel, Amer. J. Physiol. 1925, *74*, 639.
Ivy, A. C., M. Hanson and M. I. Grossman, Abstr. XVII. Internat. Physiol. Congr. 1947, 323.
Ivy, A. C. and A. J. Javois, Amer. J. Physiol. 1924, *71*, 604.
Kaijser, K., *Magsaftundersökningar på barn*, Stockholm 1943. (Swedish.)
Kaiser, P., Schweiz. Z. allg. Path. u. Bakt. 1939, *2*, 3.
Kasanki, N., Jahresbericht ü. d. Thier-Chemie 1903, *33*, 552.
Katsch, G. and H. Kalk, Klin. Wochenschr. 1926, *5*, 1119.
Keeton, R. W., F. C. Koch and A. B. Luckhardt, Amer. J. Physiol. 1920, *51*, 454.
Keeton, R. W., A. B. Luckhardt and F. C. Koch, Amer. J. Physiol. 1920, *51*, 469.
Klein, E. and E. Arnheim, Arch. Surg. 1932, *25*, 433.
Kobayashi, K., Acta scholae med. Univ. Kyoto 1926, *8*, 465.
Kolm, R., S. A. Komarov and H. Shay, Gastroenterology 1945, *5*, 302.
Komarov, S. A., Proc. Soc. exp. Biol. Med. 1938, *38*, 514.
Kosaka, T. and R. K. S. Lim, Proc. Soc. exp. Biol. Med. 1930, *27*, 890.
Koskowski, W., C. r. Acad. Sci., Paris 1922, *174*, 247.
Le Play, A., *Technique opératoire physiologique — Tube digestif et ses annexes*, Paris 1912.
Lim, R. K. S., A. C. Ivy and J. E. McCarthy, Quart. J. exp. Physiol. 1925, *15*, 13.
Linde, S. and T. Teorell, personal communication.
Linde, S., T. Teorell and K. J. Öbrink, Acta Physiol. Scand. 1947, *14*, 220.
Lindgren, G., Acta Physiol. Scand. 1943, *6*, 286.
Lips, A. C. M., J. C. M. Verschure and Th. Strengers, Acta Med. Scand. 1947, *129*, 274.
Mahlo, A., Der Magenschleim, Stuttgart 1938.
Maruno, Y., In Matsuo: Biol. Unders. ü. Farbstoffe 1935, vol. II, p. 520.
Matsuo, I., *Biologische Untersuchungen über Farbstoffe*, Vol. I and II, Kyoto 1934—35.
McCarell, J. D. and C. K. Drinker, Amer. J. Physiol. 1941, *133*, 64.
McElin, T. W. and B. T. Horton, Gastroenterology 1946, *7*, 100.
Morrison, S., Amer. J. Dig. Dis. 1938, *5*, 617.

Nielsen, H., *Forelæsninger over klinisk endokrinologi*, vol. II, Copenhagen 1941. (Danish.)
Nilzén, Å., Acta Dermato-venereolog. 1947, *27*, Suppl. 17.
Nordenfelt, P. J. and T. Teorell, Acta Med. Scand. 1935, *85*, 525.
Pavlov, J. P., *Die Arbeit der Verdauungsdrüsen*, Wiesbaden 1898.
Penner, A., F. Hollander and A. Post, Amer. J. Dig. Dis. 1940, *7*, 202.
Popielski, L., Pflüg. Arch. ges. Physiol. 1920, *178*, 214.
Riggs, B. C. and W. C. Stadie, J. Biol. Chem. 1943, *150*, 463.
Rocha e Silva, M., Arg. Inst. Biol. 1942, *13*, 99.
—— J. Pharm. exp. Ther. 1943, *77*, 198.
—— J. Allergy 1944, *15*, 399.
Roseman, R., Pflüg. Arch. ges. Physiol. 1907, *118*, 467.
—— Handbuch d. norm. u. path. Physiol. 1927, *3*, 819.
Rothlin, E. and R. Gundlach, Arch. internat. Physiol. 1921, *17*, 59.
Schales, O. and S. S. Schales, Arch. Biochem. 1945, *8*, 285.
Shay, H., S. A. Komarov, H. Siplet and S. S. Fels, Science 1946, *103*, 50.
Szokolow, A., Jahresbericht ü. d. Thier-Chemie 1904, *34*, 469.
Tarras-Wahlberg, B., Skand. Arch. Physiol. 1936, *73*, Suppl. 1.
Tejima, T., In Matsuo: Biol. Unters. ü. Farbstoffe, 1935, vol. II, p. 509.
Teorell, T., Acta Med. Scand. 1928, *68*, 305
—— Plüg. Arch. ges. Physiol. 1932, *231*, 140.
—— Skand. Arch. Physiol. 1933, *66*, 225.
—— Acta Med. Scand. 1935 a, *85*, 518.
—— Proc. Nat. Acad. Sci. (Wash.) 1935 b, *21*, 152.
—— Arch. Int. Pharmacodyn. Thérapie 1937 a, *57*, 205.
—— Arch. Int. Pharmacodyn. Thérapie 1937 b, *57*, 226.
—— Skand. Arch. Physiol. 1937 c, *77*, 81.
—— J. gen. Physiol. 1937 d, *21*, 107.
—— J. gen. Physiol. 1939, *23*, 263.
—— J. Physiol. 1940, *97*, 308.
—— Gastroenterology 1947, *9*, 425.
Trevan, J. W., Proc. Roy. Soc. ser. B. 1927, *101*, 483.
Uvnäs, B., Acta Physiol. Scand. 1942, *4*, Suppl. 13.
—— Acta Physiol. Scand. 1943, *6*, 97.
—— Acta Physiol. Scand. 1945 a, *9*, 296.
—— Acta Physiol. Scand. 1945 b, *10*, 97.
Vineberg, A. M., Amer. J. Physiol. 1931, *96*, 363.
Walko, K., Jahresbericht ü. d. Thier-Chemie 1903, *33*, 552.
Webster, D. R., 1933; quoted from Babkin, B. P., Amer. J. Dig. Dis. 1938, *5*, 107.
Welin, G. and A. R. Frisk, Acta Med. Scand. 1936, *90*, 543.
West, E. S., B. E. Christensen and R. E. Rinehart, J. Biol. Chem. 1940, *132*, 681.
Widmark, E. M. P., Acta Med. Scand. 1919, *52*, 87.
Widmark, E. M. P. and J. Tandberg, Biochem. Z. 1924, *147*, 358.
Wilander, O. and G. Ågren, Biochem. Zeitschr. 1932, *250*, 489.

Wilhelmj, C. M. and S. L. Moskowitz, Amer. J. Physiol. 1932, *102*, 620.
Wilhelmj, C. M., F. T. O'Brien and F. C. Hill, Amer. J. Physiol. 1936, *115*, 5.
Winberg, H., Acta Chem. Scand. 1947, *1*, 351.
Winberg, H. and K. J. Öbrink, Acta Chem. Scand. 1948, (in press).
Wirschubski, Jahresbericht ü. d. Thier-Chemie 1900, *30*, 374.
Zimmermann, K. W., Ergebn. Physiol. 1925, *24*, 281.
Öbrink, K. J., Acta Physiol. Scand. 1946, *12*, 213.
—— Experientia 1947 a, *3*, 455.
—— Abstr. XVII. Internat. Physiol. Congr. 1947 b, p. 244.

Uppsala 1948. Almqvist & Wiksells Boktryckeri AB 48239